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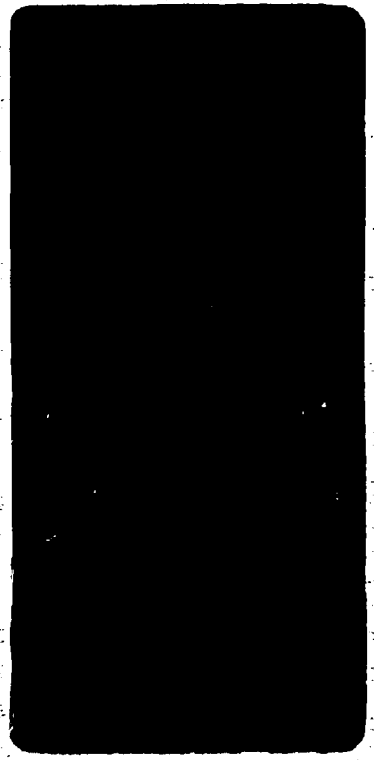

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# PHILIP MORRIS U.S.A.

## RESEARCH CENTER
## RICHMOND, VIRGINIA

## MONTHLY PROGRESS REPORTS

**Period Covered**

August 1-31, 1984

**Date Issued**

September 15, 1984

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CHARGE NUMBER: 1990

PROJECT TITLE: Blend Development

PROJECT LEADER: C. Moogalian

PERIOD COVERED: August, 1984

## I. Special Tobaccos

Objective:

To determine the character and possible utilization of tobaccos that are typical of current usage.

Status/Plans:

Evaluation of Malawi 84 crop Dark Fire-Cured samples did not show an acceptable fire-cured taste. The higher stalk samples may have utility as a Dark Air-Cured source.

We are in the process of evaluating Agrinion Djebel tobacco (semi-Oriental) in the expanded as well as unexpanded state. Evaluations should be completed in September.

In POL 2086, where a blend containing Dark Air-Cured with two menthol levels (0.41, 0.59) was tested against Salem Lights, the Salem Lights was significantly preferred over both test models by Salem Lights smokers. The Salem Lights tar value in this test was 10.2, while the low menthol model was 5.5 and high menthol model was 6.6. Liking scores were similar to Salem Lights for both models. It was suggested by Lisa Eby, who reviewed the data, that a 0.50 menthol would have performed better. We plan to POL the 0.50 menthol with a change in aftercut from the B&H Menthol system to the test aftercut currently being used in Project Hercules.

Further work on a 16 mg menthol blend is continuing. Remakes of two promising blends, both containing special tobaccos, have been requested.

## II. Miscellaneous

A. POL 4330 showed Winston Lights with significant preference over the 10 mg High Bright test blend (special aftercut) among Winston Lights and Marlboro Lights smokers. Harshness in the test model was significantly higher.

B. In the improved MUL project, preliminary data from POL 4316 indicates no significant differences in the test blend and the control.

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C. In Project Premium, an improved prototype has been recommended.

D. In Project Slow, two blends have been submitted as prototypes.

E. For the improved Merit program, two blends are currently being evaluated. In addition, 4 bench models have shown promise and have been requested in Semi-Works.

C. Moogalian

/gmm

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CHARGE NUMBER: 2100

PROJECT TITLE: New Cigarette Technology

PROJECT LEADER: W. G. Houck, Jr.

PERIOD COVERED: August, 1984

## I. Project DATA

Objective:

To develop an adjustable tar cigarette product.

Status:

Completed initial extended machineability trials.

Completed pilot market research testing.

Optimizing plugwrap for menthol extended use test.

Completed initial tests of tip turner.

Plans:

| | |
|---|---|
| Optimize tip turner | Sept./Oct., 1984 |
| Complete pilot extended use menthol testing | Sept./Oct., 1984 |
| Initiate large scale extended use testing | 3rd Qtr., 1984 |
| Product optimization | On-Going |

## II. New Package Designs

Objective:

To develop new and novel package designs.

Status:

Completed Phase II consumer testing of Hercules foil wrapped packaging.

Making alternate/improved designs for Phase III testing.

Plans:

| | |
|---|---|
| Complete Phase III testing | October, 1984 |
| Complete focus group testing of novel package designs | 4th Qtr., 1984 |

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III. Project Vanguard

Objective:

To develop a consumer acceptable nonburning smoking article.

Status:

Several designs being explored (i.e., electrical heating, flash bulb heating, SWEPT devices, low/no tar burning articles).

Completed confidentiality agreement with GTE.

Initiated flavor evaluation of electrical design.

Showed prototype units to Canada and Europe.

Plans:

| | |
|---|---|
| Explore new designs. | On-Going |
| Optimization of current ideas. | On-Going |

W. G. Houck, Jr.

/gmm

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CHARGE NUMBER: 2105

PROJECT TITLE: Filter Development

PROJECT LEADER: W. A. Nichols

PERIOD COVERED: August, 1984.

I. Filter Development - FML (W. Nichols)

Objective:

Develop the technology required to manufacture filters from FML polypropylene tow.

Status:

Machine efficiency testing was performed. Two bales of tow were evaluated at 100 m/min. Efficiency was monitored with and without hardener application. Target efficiency was 50%. Results showed the following:

| | Machine Efficiency | |
|---|---|---|
| Bale No. | Hardened | Unhardened |
| T471-003 | 69% | 74.6% |
| T471-006 | 36.8% | 60.4% |

Deviation from target efficiency on bale T471-006 was caused by unusual amounts of unfibrillated fiber in the tow band which led to rollwraps on the plugmaker. A modified feed roll is being made to eliminate the problems.

Foamed adhesive application was successfully evaluated as an alternate method for fiber bonding. Less adhesive (14% vs. 25%) was required with this technique. As this method coats most of the fiber surface, subjective and analytical testing will be performed versus sprayed adhesive application.

Subjective evaluation of all qualified hardeners was performed. Results are being analyzed. Further testing will be done with Pakistan tobacco.

The feasibility of using FML material as the inner filter on a Lark dual filter is being tested. Samples are being prepared for subjective and analytical testing.

Plans:

Demonstrate acceptable process for plugmaking. September 1984

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II. Tobacco Extrusion (R. Thesing)

Objective:

To develop an extruded foam tobacco product.

Status:

Process

Both the microwave dryer and the plenum dryer were evaluated. Under the first configuration, the extrudate issued from the die, was transported with air across the plenum, and then collected for OV determinations. The plenum dryer is capable of transporting the product and reducing its OV by 1%. When the product was then passed through the microwave cavity, it issued at 19% OV (another 2% reduction).

The microwave was more efficient when the extrudate issued from the die and entered the microwave first. (OV reduction of 3.4%). A higher wattage generator (6kw) will be tested to further dry the product to the desired 15-16% OV.

The new mixer and lift mechanism has been installed and is working satisfactorily.

The second test at Baker Perkins on the Twin Screw extruder is set for the week of September 10th.

Product

Initial testing of the windup rolls and tensioning unit is underway. Efforts are being directed to produce a uniform length of the product, dry it off line, and then paper wrap and machine tip for subjective evaluations by the flavor panel.

Plans:

| | |
|---|---|
| Complete prototype extrusion line | September 1984 |
| Produce a subjectively acceptable cigarette | Continuous |

III. Menthol on Foil (G. Patron)

Objective:

To determine the commercial acceptability of mentholated pack foil.

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Status:

Mentholated foil for the 4th and final B&H Menthol POL was made. Test cigarettes were produced at a target level of 86 mg per pack.

Cigarettes were made from filler with menthol contamination at 7 and 10%. Fillers and cigarettes were submitted for menthol analysis.

A higher mechanical drive ratio was installed on the Rothmans Applicator to allow higher flow rate for MUL Menthol application. The modified drive system will now permit menthol application of up to 9 mg per cigarette using the conventional 5 3/32" x 30 lb. foil for MUL cigarettes.

The PM designed applicator was received. Electrical installation is being completed by Engineering.

Plans:

Evaluate PM Applicator September 1984

Walt Nichols

2001115244

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CHARGE NUMBER: 4009

PROJECT TITLE: Development Smoke Studies

PROJECT LEADER: B. L. Goodman

PERIOD COVERED: August, 1984

## I. PROJECT SLOW (B. Demian)

### Objectives:

Develop the technology to reduce or mask objectionable odor of sidestream smoke.
Develop a subjectively acceptable cigarette with reduced sidestream visibility.

### Status:

Data analysis was completed for the sidestream smoke sensory evaluation test conducted in cooperation with the Internal Testing Group. The panel consisted of half smokers and half nonsmokers at the Operations Center. Each panelist rated four different cigarettes monadically for odor intensity, odor pleasantness, eye and nose/throat irritation, and acceptability. The nonsmokers rated all cigarettes as being more intense in sidestream and having more nose/throat irritation than did smokers. Smokers and nonsmokers also tended to rank the models differently, although none of the models was rated significantly better overall on any of the attributes. The nonsmokers found the MgO model less irritating to the eyes than the control Marlboro Lights. Although not significant, the nonsmokers found the control to be the least acceptable while the smokers rated it the highest. The flavor development panel did not see significant differences in the descriptors used for the O/C panel, but found the control Marlboro Lights to have more ammonia than the three sidestream prototypes.

A sidestream smoke sensory evaluation test was performed for a cigarette with a simulated leaf wrapper versus a Players control. The Flavor Development sidestream smoke panel evaluated the cigarettes in a paired comparison test, where none of the seven descriptors showed any statistically significant difference.

Method development for sidestream smoke nicotine determination is in progress. Experiments showed that Cambridge pad trapping was not efficient in collecting all the nicotine in the sidestream smoke of the Canadian Passport cigarette. Double Cambridge pads impregnated with sodium bisulfate performed better, but there is indication that some of the nicotine in sidestream still is not getting trapped. Other methods for nicotine collection are being investigated.

Analysis of average particle sizes was done by the Filtration Physics group on three Canadian cigarettes, our low sidestream prototypes, and a control Marlboro Lights. Only the Marlboro Lights was different from the others with its larger particle size.

2001115245

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Plans:

Complete the analysis of sidestream smoke from Passport cigarettes.

Continue the development of low visibility and improved sidestream odor cigarettes.

Continue the search for sidestream odor modifiers, including wrapper coating techniques.

II. COMPUTER APPLICATIONS (R. Greene)

Objective:

To write programs in BASIC and provide user assistance for the directorate at O/C.

Results:

A routine was added to the DATED control chart program to write an Inunit command file for MINITAB processing. The routine writes the file with a number in column 1 corresponding to the days since the initial date and writes all descriptors in the following columns. All columns are given names corresponding to the descriptor name.

A new program, AMINO-RSD, was released to the Basic Library. This program reads files and writes the RSD calculations for any number of groups of data. Documentation for the Basic Library program RSD was released as file BLIB:RSD.DOC. Further documentation was printed in the CAD Newsletter for RSD and AMINO-RSD. Documentation in the Newsletter was also provided for several command files which have been added to the system library in CMD:.

The first of several modifications was made to the DELIVERY program to help speed data entry. An additional development version was released to debug a new internal data representation which will eventually permit a reduction of storage file size and data retrieval times.

B. Goodman

2001115246

Source: https://www.industrydocuments.ucsf.edu/docs/yrfm0000

CHARGE NUMBER: 4010

PROJECT TITLE: Cigarette Components and Smoker Studies

PROJECT LEADER: W. A. Geiszler

PERIOD COVERED: August, 1984

I. Mentholated Foil (K. Gunst)

Objective: Determine consumer acceptability of B&H Menthol 100's with menthol applied to the packaging foil.

Status: The third POL test of the menthol-on-foil prototype versus the production control cigarette is in the field and scheduled for completion in September. The cigarettes for the third test were aged six weeks prior to shipment. Cigarettes for the fourth and final test are undergoing smoking analysis.

Plans: Complete consumer testing for B&H 100's. Initiate consumer testing for Merit Ultra Lights KS Menthol.

II. Lark Filter Design (K. Gunst)

Objective: Determine the feasibility of (1) replacing SCCW carbon with MF2C carbon in Lark and Lark Milds and (2) designing a common filter for both Lark and Lark Milds.

Status/Plans: Analytical testing of Lark KS prototypes by TTG has shown no significant differences in particulate or gas phase smoke deliveries between Lark SCCW carbon (coal based, impregnated) and Parliament MF2C carbon (coconut shell based, not impregnated). MF2C carbon could offer both cost and processing advantages. Plans are being made for consumer testing of the two carbons in Lark KS.

A common filter design is being considered for Lark and Lark Milds to avoid changeovers in plug making. Computer modeling has indicated that the 21 mm filter used on Lark KS could be used on Lark Milds KS with reasonable changes in filter ventilation and paper porosity. Similarly, a common 27.5 mm filter looks feasible for the 100 mm products according to computer modeling. Cigarette models will be made to test the feasibility further.

III. Tobacco-like Cigarette Wrapper (K. Gunst)

Objective: Evaluate a tobacco-containing cigarette wrapper from Kimberly-Clark.

Status/Plans: Cigarette models were made with six brown experimental wrappers composed of 51% tobacco material, the balance being softwood fiber and calcium carbonate. The brown wrappers have 28-35 second Greiner porosities and deliver 20% more FTC tar and 50% more CO than a 16 second conventional wrapper. A second set of models designed to deliver 11 mg FTC tar have been made for evaluation.

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IV. White Tipping (K. Gunst)

Objective: Qualify Kimberly-Clark as a supplier for white tipping with >88% opacity and 0% filter flare-up rate.

Status: An 88% opacity tipping basesheet from K-C identified as GSR-236 was printed to Marlboro Lights 100's specifications and evaluated for filter flare-up. The flare-up rates were 0.2% versus 0% for the newly qualified Ecusta 30436 sheet and 6% for the current Marlboro Lights tipping basesheet (GSR-156).

Plans: Conduct machinability and taste testing for the GSR-236 tipping.

V. True Cigarette Design (W. Geiszler, R. Arthur)

Objective: Evaluate the smoking characteristics of the new True cigarette design.

Status: Flavor Panel evaluations of the new True "Flavor Chamber" filter have indicated that the new filter delivers slightly more flavor response than the old design. Since the filter ventilation levels of the old and new True KS designs respond differently to increasing flowrates, both cigarettes were smoked with 50 cc and 75 cc puff volumes on the smoking simulator. The new design gives 5-10% more TPM and one more puff than the old design at the elevated puffing volumes. Both designs deliver 5 mg FTC tar in CI smoking with 35 cc puffs.

Plans: Evaluate a dual filter design with Hauni laser ventilation on Merit Ultra Lights.

IV. Lip-Release Coatings (R. Arthur)

Objective Improve the lip-release characteristics of white tipped cigarette products.

Status: In a second Cross Complex Panel test, Marlboro Lights tippings converted by Hermetite have been rated poorer than several other white tippings in lip release properties. Samples of tippings prepared by Thiele-Engdahl, the nitrocellulose supplier, with several different formulations were screened internally, with an Ecusta formulation receiving the highest ratings.

Plans: Discuss results with Thiele-Engdahl and Hermetite, and conduct further testing.

WAG/fms

W. A. Geiszler

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CHARGE NUMBER: 4010

PROJECT TITLE: Tobacco Studies

PROJECT LEADER: H. V. Lanzillotti

PERIOD COVERED: August, 1984

I. Low Nicotine and Control (J. Hearn)

Objectives:

Identify mechanisms of nicotine delivery. Develop techniques for nicotine delivery control. Investigate subjective effects of various sources of nicotine in cigarette smoke.

Status:

Nicotine Delivery Efficiency

A reference[1] dealing with the reduction of nicotine delivery by addition of acids to filler has been recieved.

In general, transfer rate of nicotine to smoke decreases with increasing level of acid addition and inorganic acids are more efficient than organic ones at reducing transfer rates.

II. Nonvolatile Components of Tobacco (R. Hale)

Objective:

To separate, identify and subjectively evaluate nonvolatile components of Bright and Burley tobacco.

Status:

Twenty gallons of extract from Bright tobacco (200 lbs. extracted by Felton Int'l, Inc.) have been cleaned up by washing with hexane and dichloromethane.

One half (10 gallons) of the extract has been passed through carbon columns. To ensure that all of the components have been absorbed on the columns the extract will be passed through a second time. The columns will be developed with a gradient solvent system to elute the glycosidic fraction from the columns.

Plans:

To continue the clean up of the trapped components for MS and NMR identification.

To continue work on the large extraction to obtain enough components for smoke evaluation.

1- Moknachev, et. al. "IYV. VYSSH.UCHEBN.ZAVED., PISCH. TEKNOL.," 1979, (1) 71-3.

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Source: https://www.industrydocuments.ucsf.edu/docs/yrfm0000

III. Ammonia Effect (H. Lanzillotti)

Objectives:

Identify subjective contributions of ammonia in smoke.

Identify subjective effects of sources of ammonia in tobacco.

Determine optimum ammonia sources and deliveries for specific subjective effects.

Status:

Analysis of Bright tobacco cigarettes injected with ammonium hydroxide does not show losses of reducing sugars comparable to those observed in experiments where the tobacco was treated with ammonia gas.

At reduced levels of ammonium hydroxide, the peppery character of Bright tobacco is still very prevalent.

In earlier experiments, it was found that tobacco dried before impregnation with ammonia showed an increase in total nitrogen which could not be accounted for by an increase in soluble ammonia or a reduction in reducing sugars. This bright tobacco was found to be more "Burley Like" than tobaccos which were not predried.

Additional experiments show that tobaccos treated with ammonium carbamate show a greater reduction in reducing sugars, for a given increase in total nitrogen, than ammonia gas impregnated tobaccos. These samples were found to be less "Burley Like."

IV. RL (H. Lanzillotti)

Objective:

Conduct investigations with Reconstituted Tobacco Development to improve the smoking properties of RL.

Status:

RL/RCB

Cigarettes made from the below listed experiments have been weight/dilution/RTD selected and await analytical results for panel smoking.

1. Batch cooking of CEL

    a. DAP level
    b. 50/50 mix, RCB and TC flavors

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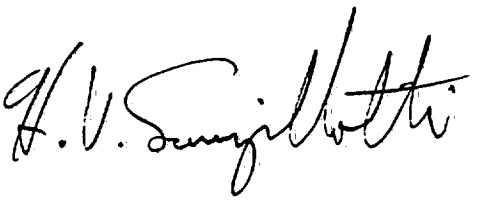
Additional experiments have been planned to optimize the Batch CEL cook and the DAP/sugar/ammonia precook processes.

These experiments will be conducted under isothermal and iso pH conditions.

Plans:

Evaluate cigarettes from recent experiments incorporating all DAP in either CEL or precook without spraying of sized sheet.



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CHARGE NUMBER: 4015

PROJECT TITLE: Brand Development

PROJECT LEADER: J. N. Smith

PERIOD COVERED: August, 1984

I. Improved Merit (L. Brown)

Objective: To improve the current production Merit 85/100 mm Regular and Menthol products to increase percent market share.

Status: The extended smoking on the cross-complex panel of 85 and 100 mm Merit with a charcoal filter is complete. The results indicated that there were no negative attributes associated with the extended smoking of the product. A more extensive extended smoking study using the POL panel has been initiated.

The HTI testing of identified and unidentified Merit and MUL with charcoal is planned. A charcoal loading of 60 mg was selected for the MUL testing.

Consumer testing of blend and flavor modifications of the current Merit product is in progress. This includes the Marlboro and Gamma systems. Testing of Merit versus competitive brands is also in progress (e.g., Winston Lights, Vantage, Marlboro Lights).

To gain more information concerning the quality and sensory parameters associated with Merit, a series of group discussions, mail surveys and telephone interviews have been initiated.

Alternatives to charcoal are being investigated. Cigarettes made with a range of silica gel loadings (0, 30, 45, 60, 75 mg) are under evaluation.

Plans:

| | |
|---|---|
| Complete HTI testing | October, 1984 |
| Complete POL extended smoking study | October, 1984 |
| Complete POL testing of Series I blend/flavor modifications | October, 1984 |
| Complete competitive brand testing | October, 1984 |

II. Project Bristol (E. Stagg)

Objective: To develop a generic/value entry product with appeal to generic and RJR smokers.

Status: The Bristol RGA testing is in progress. Analysis of test results will be complete by the end of September.

Additional in-house screening of low cost blends is proceeding. Four blends were selected for further testing.

The POL monadic testing of Doral 85 mm and the L&M Generic 85 mm is in the field.

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Project Bristol (continued)

Plans:

| | |
|---|---|
| Complete Bristol RGA | September, 1984 |
| Continue low cost blend screening | September, 1984 |
| Complete POL testing of Doral and L&M Generic | October, 1984 |

III. Project Zipper (E. Stagg)

Objective: To develop a 100 mm oval-filtered cigarette with a 23.0 mm circumference.

Status: The Zipper Test Market is in progress. Expansion to one additional test city is under consideration.

The New York Market Research extended smoking test for Zipper is complete. Data is being compiled and analyzed.

JNS:fs

J. N. Smith

J. N. Smith

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ACTIVITY: Optimum Sugar/Casings

PERIOD COVERED: July 27 - August 27, 1984

DATE OF REPORT: August 27, 1984

WRITTEN BY: B. K. Mulherin

OBJECTIVE:

To subjectively evaluate/optimize current casing systems.

To develop new or special casings for new or improved products/processes as requested.

STATUS:

A. Licorice

Subjective evaluation of cigarette models to evaluate the interchangeability of licorice flavor systems continues. Initial subjective evaluations of the 52 percent burley models were by paired comparison. Descriptors used in the initial evaluations were incorporated into a descriptive ballot for further evaluations. Cigarettes are now being smoked by a limited panel using replication of each pair. Licorice flavor systems include block licorice, spray dried licorice, and licorice replacement. Evaluation of Marlboro cigarettes using the Y84-1 blend and the licorice flavor systems is scheduled for testing by the Flavor Development Panel.

Two additional samples of licorice from new suppliers will be evaluated in Louisville for qualification to established analytical specifications. Subjective evaluation of cigarette models incorporating the spray dried sample in the casing system is in progress. Production of cigarette models for subjective evaluation of the "new" block sample will be determined after chemical analyses are completed.

Subjective monitoring of Marlboro B Test Market samples continues. No significant difference was found for soft pack cigarette samples when tested against the current production control. A significant difference was found between flip-top box test and production control.

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B. Humectant Studies/Casings and Aftercuts

The storage study of Saudi Marlboro Lights is scheduled for completion in September. The primary objective of the study was to reduce spotting and staining of cigarettes. Variables tested were a normal aftercut which incorporates the humectants PG and glycerin, and a tropical flavor formulation which did not incorporate PG and glycerin. While spotting and staining were reduced (140° F, 7 days), subjective differences were indicated by the Flavor Development Panel.

Discussions were held with the Senior Staff and Activity Members concerning objectives and direction for evaluation of alternate polyols and increased menthol solubility by manipulating humectants in casings and aftercuts. Clarification of objectives and revision of plans are in progress.

C. Humectant Studies/Whole Leaf Tobacco and Sheet Materials

A completion report on humectant application has not been received from Les Bradshaw of Tobacco Processing.

PLANS:

A. Licorice

| | |
|---|---|
| Continue monitoring of Marlboro B Test Market | On-going |
| Summarize POL's evaluating alternate method of application of licorice replacement | Sept. 1984 |
| Continue evaluation of licorice flavor systems | On-going |
| Analytical evaluation of licorice flavors from new suppliers | Sept. 1984 |

B. Humectant Studies/Casings and Aftercuts

| | |
|---|---|
| Complete Saudi Marlboro Lights Storage Study | Sept. 1984 |
| Evaluation of alternate humectants | On-going |
| Revise study for PG rearrangement/menthol solubility | Sept. 1984 |

C. Humectant Studies/Whole Leaf Tobacco and Sheet Materials

| | |
|---|---|
| Complete report on sorbitol replacement of TEG in RL and RCB | Sept. 1984 |
| Review results of humectant application to green leaf tobacco | When report available |

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B. K. Mulherin

B. K. Mulherin

BKM/abt

cc: F. L. Daylor
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CHARGE NUMBER: 0307
PROJECT TITLE: Measurements Development
PROJECT LEADER: D. R. Fox
PERIOD COVERED: August, 1984
DATE OF REPORT: September 6, 1984

## I. Loose Ends

Objective:

To develop a dynamic sensor and/or laboratory instrument for measuring loose ends and to determine the effect of particle size, particle mixing, blend formula, tobacco weight, and OV on cigarette loose ends.

Status:

A second set of blends and cigarettes have been requested from Semiworks to study the effect of DIET addition on loose ends and the mixing law relationship for loose ends. The first test, in which DIET was mixed with DBC bright tobacco, showed increasing loose ends with higher levels of DIET at constant firmness and density index. The second test will again involve mixtures of bright and DIET. Two types of DIET will be used: normal production DIET, and pilot plant DIET from Project 1801 that has significantly improved shred size.

Plans:

The second DIET-bright test is expected to be run later in September.

## II. Tobacco Moisture

Objective:

To develop an instantaneous, non-destructive technique for determining the moisture content of bulk tobacco and cigarettes.

Status:

Calibrations for Marlboro filler were obtained from on-line testing in Semiworks, in which cutter and dryer conditions were varied to give a range of moistures, as well as from laboratory conditioning of material. The data from the various methods compare well to each other at higher moistures. At lower moistures (below ~13%), there was a difference in response between the samples tested "on the fly" during the Semiworks run and the laboratory samples. Since PG and Glycerine analyses showed no significant difference between the two sets of samples, tobacco temperature is suspected to be the cause. Tests are planned to investigate this and to try to differentiate between actual instrument sensitivity effects and possible changes in sample moisture due to evaporative cooling.

Since calibrations for Marlboro have been installed for the OC Semiworks unit and several procedural problems have been corrected, good results have been obtained. The Richmond Stemmery started up during the month and has reported good results with their new units. Based on their results, the Louisville Stemmery has decided to purchase an L-band unit immediately rather than

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borrow one from Richmond for evaluation.

An IBM-PC XT was interfaced with the L-band in the lab to store microwave results in a form that can easily be transferred to the DECsystem. The STSC APL package on the PC was used for this task. The APL is quite versatile and powerful, and required very little time to program. As a result, we will be using the PC/APL system for other tasks in the laboratory in the future.

Plans:

Additional calibrations will be developed in the lab for other materials, including for several grades of bright tobacco to be used in a test to evaluate the effect of large stem level on cigarette properties (to be conducted by Project 0400). Testing on the effect of tobacco temperature will also be conducted to determine whether it is the cause of the offsets observed between on-line and lab calibrations for low OV's. IBM PC's are expected to arrive early in the month for the Semiworks units, and these will be programmed and installed.

III. Mathematical Modeling

Objective:

To update the tobacco models for filling power and cigarette firmness to incorporate new tobacco materials and new measurement methods.

Status:

Testing was completed for the Blend Component Study and Model Update. Tables for the BCS were completed and are currently being reviewed. While the CV/OV data is quite reasonable, some of the sieve data shows a good deal of variability. This is not unexpected for the sieve test, but since the BCS is used as a reference, a decision on how best to report the sieve data will have to be made.

Plans:

The Blend Component Study should be submitted for approvals during September. Work will then begin on generating the mathematical models for CV, OV, RH, and firmness.

D. R. Fox

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CHARGE NUMBER: 0400
PROJECT TITLE: Tobacco Properties Applications
WRITTEN BY: W. T. Callaham and R. S. Mullins
PERIOD COVERED: August, 1984
DATE OF REPORT: September 7, 1984

## I. Whole Leaf Cutting

Objective:

Develop a process for cutting and separating whole leaf into cigarette filler.

Status:

The installation of new combing roll slats on the primary carding feeder has resulted in an approximate 10% increase in product 6 mesh size with no change in product stem content or yield. The new comb which was installed just prior to the straight-laying fancy roll had no effect on VLS product size, stem content or yield, therefore, it will not be used in future testing.

Further studies of the VLS separator during the latter stages of carding have indicated a poor separation between filler and stems compared to the VT separator. However, use of the Cardwell Stem Tester after three carding stages has been more promising. This has resulted in a clean filler/stem separation and high lamina recovery; but, at the expense of a moderate increase in the 35 mesh and dust fractions of the product. The CST will continue to be investigated.

Preliminary studies were conducted to determine the applicability of either an expansion tower or a steam tunnel to declump/disentangle exit cutter whole cut leaf. The tower worked well and this concept will be explored further. The steam tunnel tended to further entangle the filler.

Several preliminary studies were conducted on utilizing the cut stems for shredded stem production via the Sprout-Waldron and the Quester refiners. The Quester refiner gave a better looking product with CVs ranging from 5.5cc/g (as-is) to 8.7cc/g (expanded). After a couple more definition runs, a larger trial will be made comparing this product with RL-TC made from cut stems.

Whole cut leaf fillers were generated for a cigarette stem hole study and a comparison study against threshed leaf fillers. The cigarettes will be made and analyzed in September.

Finally, an order was placed with the Leaf Department for 98,600 pounds of a quasi-DBC leaf blend to be used for whole leaf and strip testing. This tobacco will be available by the end of October.

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PLANS:

Complete the stem hole study and the comparison study, conduct another filler reblending study, evaluate the effect of casings on carding/separation, investigate the use of notched cutter blades and fine cutting (90CPI), and evaluate the secondary carding feeder in front of the VT separator.

## II. Maker Optimization Program

Objective:

Optimize cigarette makers to reduce degradation of filler and improve product quality.

Status:

Additional testing has confirmed that installation of the fancy elevator doffer on the belt-feed hopper reduced the cigarette weight standard deviation to approximately 40 mg. without substantially increasing filler degradation. Cigarettes produced with the fancy doffer installed continued to contain 4 to 5 percent filler greater than 6 mesh in size.

A second test was conducted to investigate the effect on filler degredation and cigarette weight variation of removing the combs which hold the tobacco on the lower carding drum just prior to the picker roller. Cigarettes produced with the combs removed contained 0.6% (absolute) more 6 mesh filler than cigarettes produced with the combs installed (2.9% vs 2.3%). No difference was detected in the weight variation of the cigarettes. Additional testing is needed, however, to confirm that the combs can be removed without increasing weight variation. Since short filler is more likely to fall out of the carding drum pins if the combs are removed, and thereby increase cigarette weight variation, the effects of removing the combs when using short filler also need to be investigated.

Plans:

Collect baseline data on the belt-feed hopper with the fancy doffer installed. Fabricate a carding feeder, similar to those used in whole leaf cutting, to more uniformly feed filler to the belt-feed hopper. Continue to investigate the effect of removing the carding combs on filler degradation and cigarette weight variation.

Robert A. Mullins

W K Callahan

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CHARGE NUMBER: 1005
PROJECT TITLE: Primary Process Development
PROJECT LEADER: R. T. Gaudlitz
PERIOD COVERED: August, 1984
DATE OF REPORT: September 6, 1984

Title: Casing Reconstituted Tobacco with Bright and Oriental (J. Nguyen)

Objective: Determine if casing recon with bright and Oriental will yield an improvement in cut filler sieves.

Status: The Marlboro formula was used to prorate the casing of bright, Oriental and recon portions of tobacco exiting the storage silos. However, no burley strip was added to either the control or test runs to accentuate results. The volume of bright casing used was maintained at the same level for both runs. Sieve samples taken at the cutter exit and Adt dryer outlet indicated that the test run gave lower particle sizes, e.g., 31.7% vs 34.2% on six mesh and 60.2% vs 62.4% on 6+12 mesh at the cutter exit, and 18.2% vs 21.3% on six mexh and 52.8% vs 56.7% on 6+12 mesh at the Adt dryer outlet.

Plans: No further work is planned.

Title: On-line Moisture Meter Improvement (J. Nguyen)

Objective: Improve accuracy of the on-line moisture measurements in the Semiworks.

Status: An extensive run with Marlboro blend was made for calibrating the on-line Quadra Beam II (QB-II) at the Adt dryer outlet. A total of 156 samples was taken in the rang eof 6.5% to 20% OV. The regression analyses indicated a strong correlation with an R coefficience of 0.990 and a standard deviation of 0.367.

Plans:
1) Continue reliability work on QB-II.
2) In conjunction with the on-line QB-II an off-line (static and semi-dynamic modes) QB-II was set up. It will be tested for measuring OV's at many different locations in the Semiworks and/or "D" primary.

Title: Cutting Study (T. Skidmore)

Objective: Using the small scale cutter determine the effect of cutting temperature, moisture, and pressure on cut filler cylinder volume.

Status: Statistical analysis of data from the recent test is practically complete. The data indicated that the best conditions for CCV and sieves were as follows:

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| | Cutting OV (%) | Cutting Temp. (F) | Cheese Density (g/cc) |
|---|---|---|---|
| CCV | 20.5 | 80 | 0.8 |
| On 6+12 | 24.6 | No Effect | 0.9 |

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It is interesting that the best cheese densities were on the lower end of the range studied (0.8 - 1.2 g/cc) suggesting that further improvement, particularly in CCV, may be possible with lower densities (cutting pressure). In the range of cutting temperature studied (80 - 150°F) increasing the temperature had little effect on shred size but had a negative effect on CCV. The data suggest that a cutting temperature below 80°F could improve CCV.

Plans: The Semiworks RC-3 and RC-4 cutters will be used to further investigate lower cheese densities. Additionally, cheese density measurements will be made on Stockton Street and MC cutters for comparison to R&D results.

Title: Steam Tunnel Development (D. Albertson)

Objective: To evaluate the effect of elevating the temperature of cut rag in a steam environment as it relates to the elimination of cutter cheese clumps prior to drying and to evaluate the potential for improvement in the physical properties of treated cut filler.

Status: The steam tunnel was modified in August to provide more steam holes in the entrance end of the unit so that a lower steam pressure could be used. It had been hypothesized that the high velocity steam at the nozzles, when operating at 100 psig, was causing degrdation to the filler resulting in a 3-5 unit loss of 6+12 mesh sieve fraction. With the additional holes in the steam plenum the pressure was reduced to 20 psig with good preliminary results. Testing with this arrangement will continue in September.

R. T. Gandhi

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PROJECT NUMBER: 1307

PROJECT TITLE : Reconstituted Tobacco Development

PERIOD COVERED: August 1-31, 1984

PROJECT LEADER: R. G. Uhl

## I. IMPROVED SHEET PROPERTIES (R. M. Rogers)

### A. Objective

Improve the physical characteristics and blend performance of reconstituted sheet.

### B. Status

1. Survivability studies of chemically traced Park 500 and pilot sheets show a definite increase in RL piece size at the maker exit as sheet tensile strength is increased. This was primarily determined by $TiO_2$ tracer analysis of sieve fractions, but was confirmed by analysis for paraben co-tracers, picking of sieve fractions and higher longs content in the actual ripped cigarettes. RL shred size distribution in 17% and 50% blends was surprisingly close to 100% RL results, indicating little in the way of synergistic effects.

2. Separate R&D and Park 500 evaluation of test sheets from pilot plant processing of Park 500 stock showed the same progression of tensile values for the eight tests. Reducing headbox consistency without additional refining gave no benefit due to formation defects from flocculation of the long stock. When additional refining was also utilized there was a definite improvement in tensile values (>50%). Data analysis indicates that large unrefined fibers have a considerable detrimental effect on tensile values. Fines have a minor effect, essentially acting as a filler to dilute tensile strength. Pilot processed sheets also show tensile correlation with basis weight.

### C. Plans

Conduct an extended pilot run with Park 500 stock at best process conditions and evaluate product for survivability and effect on cigarette properties.

## II. SUBJECTIVE MODIFICATION OF RL (R. Ellis)

### A. Objective

Improve or modify the subjective character of RL.

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B. Status

1. Subjective evaluation of up to 4% menthol CT in pilot RLTC and 150B showed no significant difference from control blend. However, the CT utilized was low in menthol (2300 vs 4500 ppm average, 6500 ppm max). Therefore, this testing only qualifies 2% usage of mentholated CT with a normal menthol content.

2. Cigarettes containing RL/RCB sheet made at high DAP levels, etc., have been submitted to the large Flavor Development Panel for clarification of preliminary results. Other models are in testing to determine the effect of sugar level and establish the equivalence of DAP spraying vs size addition.

3. The best RL/RCB variants (both CEL cooks and precooked flavors) have been made into cigarettes using the same master strip batch for head-to-head comparison. These include 7% models replacing RCB.

4. While RL/RCB provides RCB flavor notes it retains some characteristic RL qualities, resulting in a product that is more like a blend of the two production sheets. An approach is being formulated to determine what blend it may be equivalent to.

C. Plans

Expedite comparison of the two RL/RCB approaches, optimize precook flavor and DAP levels in the precooked variant, and initiate determination of RL/RCB equivalency to blends of RL and RCB.

III. RL COATER DEVELOPMENT (R. M. Rogers)

A. Objective

Develop a process for the application of class tobaccos to RL utilizing coating technology.

B. Status

1. Coated and sized RLTC made for external panel tests meet specifications and are being subjectively screened.

2. The PM Engineering Conditioner Group has improved coated RL unpacking by using 4-5 conditioner cycles while pulsing conditioner vacuum/pressure to loosen the tobacco. Pad counts for coated sheet are down to 2-4% compared to 0.6% for Park 500 sized sheet. All pads are relatively small (no tombstones) and should eventually break-up if recycled.

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C. Plans

Expedite panel testing of coated vs sized sheet. Produce RL coated with RCB slurry for subjective evaluation.

IV. RCB (R. Ellis)

A. Objective

Develop and evaluate process and product improvements for cast sheets.

B. Status

1. Comparison of U.S. and Venezuelan (BATANA) RCB was completed. The BATANA product is higher in solubles but contains less humectant and equilibrates at a lower OV. Equilibrated CV is also lower as measured, but is equivalent when corrected to an equal fiber basis.

2. RCB handsheets of black tobacco byproducts were made for subjective evaluation by TTG. Other handsheets were made for simulated storage studies of RCB and TMCI materials.

3. Several RCB test runs were conducted on the pilot Sandvik system. Problems center on steam line pluggage in the dryer section, single pass refining and controlling coating weight at the very low line speeds.

C. Plans

Continue debugging the pilot coater/belt system.

R. L. Ehl

/dbb

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CHARGE NUMBER: 1503
PROJECT TITLE: MODIFIED SMOKING MATERIALS
PERIOD COVERED: August, 1984
PROJECT LEADER: J. G. Nepomuceno

## I. TOBACCO EXTRUSION

OBJECTIVE:
Develop a process for extrusion of foamed tobacco articles.

STATUS:

Post extrusion equipment for handling and drying the extrudate were evaluated. Minimal drop in OV was observed across the air plenum; however, a significant drop in OV was observed across the 2.5 KW microwave unit. Based on these results, a 6 KW microwave generator has been ordered for evaluation.

The windup rolls were also evaluated. Some extrudate deformation was observed on the multistrand product; however, a significant amount of deformation was observed on the single strand product due to the windup mechanism. Modifications to this windup device is in progress.

A twin screw extruder from Baker-Perkins was evaluated. Results showed that product with moisture levels as low as 10% OV were achieved. Prehydration of the binders prior to mixing with the tobacco was also found to produce low OV product.

PLANS:

Continue development of post extrusion handling and drying equipment.

Further testing on the Baker-Perkins extruder is planned. Such an extruder is available for rental and/or purchase in January, 1985.

## II. FILLER BINDER

OBJECTIVE:

Develop a process for applying a subjectively acceptable foamed binder to the tobacco filler rod at the maker in order to improve coal strength, reduce loose ends and allow for rod weight reduction.

STATUS:

Application of a 13% solution of extra cellular material (ECM) to the cigarette at 0.5% level was shown to significantly improve loose ends firmness and coal strength. These cigarettes were also found to be subjectively acceptable by a smoking panel.

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Licorice was found to be an efficient foaming agent as a replacement for sodium lauryl sulfate. Subjective screening of various gums is planned using licorice as a foaming agent.

PLANS:

Further tests evaluating ECM is a foamed binder are planned. Large scale production of ECM is being explored.

Use of tobacco pectin as a binder is also being investigated. A program for extraction of the pectin from scrap tobacco is being formulated.

## III. DRY FORMING

OBJECTIVE:

Evaluate dry forming processes for making smoking articles and cigarette components from tobacco, tobacco by-products or non-tobacco materials.

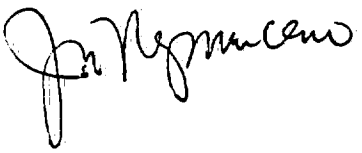
STATUS:

A high pressure sprayer was installed and was found to be capable of atomizing and applying 8% pectin solutions to the dry formed mats.

Cigarettes made from dry formed mats containing 30% stem fines showed marked improvement in cigarette firmness at 550 mg level. These fines are probably bridging across the tobacco shreds forming a more rigid and thus firmer network.

PLANS:

Continue development of dry forming process with present unit. Evaluate alternative dry forming units for production of low density webs.



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CHARGE NUMBER : 1801

PROJECT TITLE : Expanded Tobacco Development

PERIOD COVERED: August 1-31, 1984

PROJECT LEADER: G. Gellatly

## I. $CO_2$ IMPREGNATION

### A. Objective

To find means of $CO_2$ impregnation of tobacco without clumping.

### B. Status

Cabarrus developmental trials for clump-free DIET process definition by liquid $CO_2$ impregnation were completed. No greater tobacco bed compaction which causes clumping, was observed by liquid spray deflection onto the bed or direct spray through a wire mesh or through a sintered metal nozzle than is observed by gaseous impregnation.

A comparison of clump-free DIET filler made in the pilot plant with MC DET showed an improvement of 5% longs in 100% DIET cigarettes and associated iprovement of coal strength and loose ends. These improvements were not observed in 30% DIET blend cigarettes.

### C. Plans

1. Analyze results of the Cabarrus trials and make recommendations for future factory trials.

2. Complete the modification of the pilot plant to eliminate the clumpbreaker and vibrabin and demonstrate DIET longs increase by $CO_2$ spray impregnation and conveyor reordering.

3. Evaluate means of impregnation of tobacco bed depths greater than 30" in the pilot plant.

## II. STRIP OPENING

### A. Objective

To evaluate processing of opened strip for improved filler length and cigarette quality.

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B. Status

DBC control and "opened" strip was cut at moisture levels of 19% to 24% OV and the degradation of filler length through cutting, flavor application and cigarette making was measured. At low cutting OV the control filler was more susceptible to breakage than the flat filler. At a cutting OV of 22% the absolute increase in the longs content (+6 mesh) over control was 10% after the cutter and up to 5% after the maker. This longer filler translated into improved cigarette quallity. Loose ends and coal strength improved by about 1/3 in 100% DBC cigarettes. This improvement was particularly noticable at lower cigarette weights (7 weights tested). The filling power of test and control cigarettes was the same.

Cutter trials were run which successfully established operating conditions for cutting "opened" strip which had initially shown some unevenness in cut width.

C. Plans

1. Compare the surface and fiber configuration of "open" and control filler microscopically.

2. Compare the length of different sieve fractions by image analysis.

3. Evaluate the effect of cylinder reordering and drying on "open" strip.

4. Prepare a development program for this project.

/dbb

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CHARGE NUMBER: 1806

PROJECT TITLE: New Tobacco Processes

PROJECT LEADER: C. L. Irving

PERIOD COVERED: August, 1984

DATE OF REPORT: September 5, 1984

## I. STEM PROCESSING

### A. Objective

To develop a process for direct conversion of stem materials into useable filler.

### B. Status

A comparison of ES, IS, superheated IS (SIS), and superheated CRS (SCRS) was conducted to determine the effects on cigarette quality. Analysis of the cylinder volume data of the stem materials showed the SCRS to be slightly higher than the IS (5.5 vs 5.3 cc/g), while the CV of the SIS approached that of the ES (6.1 vs 6.4 cc/g). Comparisons in blends at 12% could only be made with the ES, IS, and SIS, since the cutting OV of the remainder of the SCRS blend was significantly higher than the other blends (23.2 vs 21.7%). The firmness of the blend with the SIS product approached that of the ES blend, and the SIS blend showed a higher longs fraction exit the maker (5.2 vs 3.3%). There was no improvement in loose ends however. The SIS blend did cause an increase in maker winnowers, probably due to the large size of some of the stem particles after superheating. Samples have been submitted for subjective evaluation.

A series of tests was run to evaluate the effects of stem moisture and tower steam concentration on the superheating CV boost of CRS. These tests, completed in the 3" tower with 0, 30, 50, and 100% steam, showed that the amount of expansion was directly proportional to the amount of steam present in the tower atmosphere and to the OV of the material fed to the tower. For 100% steam tower conditions the CV boost ranged from 0.07 to 0.89cc/g for feed OV's of 14 to 50%, while at 50% feed OV the CV boost ranged from -0.06 (attrition) to 0.89 cc/g for 0 to 100% steam tower conditions. Plots of the data show the impact of steam concentration to be greater the higher the input moisture. Apparently, the CV boost is entirely due to expansion (SV changes) as no changes in equilibrium OV were observed.

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A comparison of shredded products made from the old and new ES feed material (VSTKB and BST, respectively) was conducted using both the Quester and Sprout-Waldron refiners. In addition, a series of superheating/expansion runs was completed in the 3" tower at temperatures of 300, 450, and 600°F using products from both refiners. Without superheating or expansion, the Quester shredder appeared to produce the better shredded stem product for both blends (CV = 6.0 vs 5.6 cc/g). The heat treatment runs showed that better expansion (9.3 vs 8.4 cc/g) was obtained with the VSTKB than with the BST. Also, at the same tower temperature, 100% steam produced a higher CV value than 75% steam for both blends. Subjectively, shredded products made from the two blends were different (VSTKB vs BST), as were products from the same blend shredded on different refiners (Quester vs Sprout-Waldron). Expansion of the products did not eliminate these differences completely.

To determine the effects on cigarette quality, a comparison of shredded stem from the Sprout-Waldron with and without fines (11.2 vs 5.7%) was conducted. Analyses of results from testing cigarettes containing 27% shredded stem showed that the fines did not contribute to increased loose ends or have an impact on firmness. There was an increase in maker dust (2.5 vs 1.9%), as well as an increase in the pan fraction exit the garniture (3.4 vs 2.1%) for the with and without fines blends, respectively.

Production of shredded stem from each of three scrap stem materials and a blend of these materials was completed for personnel from B&H Canada. This was conducted to provide a check on the type of product that they will produce upon the installation of their shredded stem line.

C. Plans

Conduct a comparison test to evaluate the quality of rolled/cut stems vs shredded stem with various post treatments.

Evaluate the results from the test conducted at Hauni using the master batch of bright stems in the HT tunnel.

## II. MECHANISM STUDIES

A. Objective

To determine the mechanisms that are important to the processing of tobacco, such as attrition, drying, fluidization, etc.

B. Status

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Evaluation of test results from the Wolverine dryer using ambient air for fluidization of cut filler showed an 4.5% loss in material >12 mesh. This is consistent with previous drying tests, perhaps confirming that the method of fluidization in the unit is not gentle enough. In addition, product carryover into the cyclones has been 3-4% at throughputs of 400-800 lb/hr.

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Problems with the metering tube feed system have prevented proper evaluation of the moisture uniformity and may have contributed to the carryover. This led to the installation of a weighbelt feed system that will permit a more thorough evaluation of product breakage, carryover, and moisture uniformity.

C. Plans

Should previous attrition results continue, investigate alternate fluidization tube sizes for the Wolverine dryer.

Set up the Legg dryer test rig in D Pilot Plant for future batch cylinder attrition studies.

S. R. Wagoner

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PROJECT CHARGE: 1703

PROJECT TITLE: CIGARETTE MAKING TECHNOLOGY

PROJECT LEADER: A. Robinson

PERIOD COVERED: August 1 - 31, 1984

DATE OF REPORT: September 7, 1984

FRICTION PROPERTIES OF PACKAGING FOILS (A. Robinson, V. P. Henderson)

An extensive study has been undertaken in collaboration with Manufacturing and QA personnel to ascertain the surface characteristics of cigarette packaging inner wrap aluminum foils. In particular, studies have been performed to determine the surface roughness, levels of static charge and the slip-stick characteristics of the foils from PM's three principal suppliers (Alcoa, Golden Belt and Reynolds). Dynamic kinetic coefficient of friction results for the Alcoa foils, which are known to contain a dip additive, showed indeed it was easier to machine. The COF values were in the range of .24-.27 as compared to .70-.75 for the other products.

The study is being expanded to include "dead fold" (i.e., flexibility) characteristics of the foils, which is an associated machining problem.

TOBACCO ADHESIVE STUDIES (T. E. Majewski, A. Robinson, V. P. Henderson)

A parallel plate rheometer as an accessory to our Thermomechanical Analyzer (TMA) has been received and work is under way to obtain viscosity and wall shear-rate data for various extruded rod binder compositions as a function of processing temperatures.

Rheological examinations on dextrans and Klucel formulations continue on the Haake Rotovisco viscometer to establish a processability profile as a function of molecular weight and/or percent composition.

MISCELLANEOUS

A literature search has been made and a program is being initiated to develop methods of adhering polypropylene materials.

A. Robinson

/ev

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PROJECT CHARGE: 1704

PROJECT TITLE: SUPERCRITICAL FLUID PROCESSES

PROJECT LEADER: J. R. Patrick

WRITTEN BY: A. T. Hill

PERIOD COVERED: August 1 - 31, 1984

DATE OF REPORT: August 31, 1984

The supercritical screening system was modified to allow changes from extractions to depositions to be done more quickly. Also, a pumped cleaning system was built and put into place.

Samples of Oriental tobacco (Thailand, 1983) and Turkish, 1981, were furnished by Allen Palmer for extraction by supercritical $CO_2$. The tobaccos had been cured and threshed. Both extractives, their solutes and the controls were returned to Allen Palmer for analyses.

A preliminary deposition of polymenthol carbonate on Marlboro filler has been attempted and results are pending.

With the help of PM USA Engineering, Purchasing and R&D Plant Engineering and Electrical Engineering a comprehensive set of operational specifications were derived for our proposed supercritical fluids facility. Several visits to potential engineering contractors materially increased our awareness of current technology. Bids for engineering and construction of the facility will be reviewed shortly.

A. T. Hill

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CHARGE NUMBER: 1708

PROJECT TITLE: Physical and Chemical Properties of Tobacco

PERIOD COVERED: August 1-31, 1984

PROJECT LEADER: H.A. Hartung

DATE OF REPORT: August 4, 1984

## Mechanical Properties of Tobacco:

Stress relaxation experiments were carried out on the Instron testing machine to compare expanded burley with expanded bright. Unexpanded samples of the same tobaccos were used as controls. Expansion increased the pressure-volume response, of course, but it did not change the relaxation spectrum. Burley showed much less tendency to relax than bright whether expanded or not.

Relaxation tests were also conducted on filler treated with a foam binder. The binder had no effect on either the pressure or the relaxation rates. (Reported by M.E. Counts)

## Effect of Bed Depth on Reordering Kinetics:

Measurements of OV and CV as a function of time have been made using bright tobacco. It was first equilibrated at 40%Rh and then moved to 70% for the test. The beds were 4 inches deep with screen separations one inch apart so that the effect of bed depth could be closely monitored. Thus far the OV and CV changes have shown essentially the same rate constants within the same layers. The interior layers changed about four times slower than the top layer. (Reported by M.J. Wood)

## Solids in Casing Solutions:

We have started testing the hypothesis that the effective dry solids of casing solutions depends upon mixing effects with the substrate. If verified, this will reduce some of the systematic losses that are seen in the tobacco accountability system installed in the primary. We are running standard OV tests using tobaccos sprayed with carefully weighed quantities of casings. The effective dry solids of the casings are determined by comparing the residual dry weights with and without the additives.

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Source: https://www.industrydocuments.ucsf.edu/docs/yrfm0000

In the first round of tests the casings were applied at about the same low levels as normally used in the factories. The results had large random deviations because the effective dry solids were derived as small differences between large numbers. The tests are being repeated with larger than normal application levels with the hope that this will give results with good confidence limits. (Reported by L.M. Trentham)

Reports

H.A. Hartung, "An APL-Database System that Evolved in an R&D Environment" draft manuscript prepared for publication.

M.E. Counts, "Stress-Relaxation of Binder-Treated Filler" memorandum to Jose Nepomuceno, August 30, 1984.

H A Hartung

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Source: https://www.industrydocuments.ucsf.edu/docs/yrfm0000

CHARGE NUMBER: Project 1720

PROJECT TITLE: Physiochemical Morphology Research

PERIOD COVERED: August 1-31, 1984

PROJECT LEADER: C.E. Thomas

DATE OF REPORT: August 30, 1984

## I. Low Temperature Microanalysis

Tobacco samples were collected from the Southern Piedmont Station at Blackstone, Virginia. Ripe and senescent midstalk leaves of burley (Va. 504), sun cured, and bright (Coker 319) were de-stemmed and dried for elemental analysis. Fresh specimens of these samples were also frozen in liquid nitrogen for subsequent EDS analysis as frozen hydrated specimens. EDS spectra were collected from frozen dried specimens of compariable greenhouse tobaccos. Spectra were also collected from a $K^+/p$ standard with a synthetic biological matrix. Relative intensities from this standard will be used to correct for the masking effects of the chromium sample coating.

## II. Digital Image Analysis

A program has been written to calculate the size of a tobacco leaf from an image of the leaf produced by a video camera. The system can calculate leaf area, length, width, and number of holes. Another program has been written to measure the length and number of tobacco particles in cut filler. Specifically, the tobacco filler from a single Marlboro 85mm was studied. The total tobacco object count was 1208 pieces with a mean fiber length of 4.48mm. There were 1050 objects shorter than the mean length and they consisted mostly of fines with a diameter of 1mm or less. The longest tobacco shred was 48mm.

## III. Reports, Publications:


1. Baliga, V.L.; Counts, M.E., "Identification of Calcium Oxalate Crystals in Dried or Fixed Tobacco Leaf," Proceeding of 42nd Ann. Electron Microscopy Society of America, (G.W. Bailey, Ed.) p. 288, 1984 (Presented Internally August 2, 1984).

2. Echlin, P.; McKoon, M.; Taylor, S.E.; Thomas, C.E.; Maloney, K.; Hayes, T.L.; "Bulk Standards for Low Temperature Biological X-ray Microanalysis "Proceeding of 42nd Ann Electron Microscopy Society of America (G.W. Bailey, Ed.) p. 282, 1984.

3. Owens, A.; "July Daily Light Intensity Averages - Greenhouse," Memo to E. Thomas, August 15, 1984.


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Source: https://www.industrydocuments.ucsf.edu/docs/yrfm0000

4. Taylor, S.E.; Echlin, P.; McKoon, M.; Hayes, T.L.; "Low Temperature X-ray Microanalysis of Frozen-Hydrated Tobacco Leaves" Proceeding of 42nd Ann Electron Microscopy Society of America (G.W. Bailey, Ed.) p. 284, 1984. (Presented internally August 9, 1984)

5. Thomas, C.E.; "Puff by Puff Mainstream and Sidestream Tar Deliveries of Passport 84mm and Passport 72mm Cigarettes" Memo to B. Kuhn, July 23, 1984 (Also presented at monthly development meeting August 8, 1984).

C.E. Thomas

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Source: https://www.industrydocuments.ucsf.edu/docs/yrfm0000

CHARGE NUMBER: Project 1730

PROJECT TITLE: Plant Tissue Research

PERIOD COVERED: August 1-31, 1984

PROJECT LEADER: L. Weissbecker

DATE OF REPORT: August 29, 1984

## I. TOBACCO CELLS:

Cells generated from callus tissue obtained from leaf discs have been grown in suspension culture. The callus tissue from leaves produced alkaloids when subjected to certain growth regulators. Alkaloid production was monitored with Dragondorff reagent.

## II. PLANT PROTOPLASTS:

Some plant protoplasts, obtained from tissue culture grown plantlets, have undergone a few cell divisions and are being cultured for callus formation. No viable protoplasts have yet been obtained from greenhouse grown plants.

## III. GRAFTED TOBACCO PLANTS:

The first primings have been obtained of Coker 319 tobacco plants grafted onto Coker 319 roots and onto TI516 roots (a low alkaloid producer). Preliminary results from General Analytical indicate a significant reduction in the alkaloid content in flue-cured leaves from Coker 319 grafted onto TI516 roots compared to leaves from Coker 319 grafted onto Coker 319 roots. The second primings have just been received.

## IV. MISCELLANEOUS:

Tobacco tissue culture samples were sent to Dr. Ed Kot of Miller Brewing to enable them to restart their tissue culture experimentation.

## V. REFERENCES:

Karen Barlow - notebook 8061.
Maria Shulleeta - notebook 8099.
Lou Weissbecker - notebook 7925.

L Weissbecker

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Source: https://www.industrydocuments.ucsf.edu/docs/yrfm0000

CHARGE NUMBER: Project 1901

PROJECT TITLE: Biochemical Modification of Tobacco

PERIOD COVERED: August 1-31, 1984

PROJECT LEADER: D. M. Teng

DATE OF REPORT: August 30, 1984

I. Curing Study:

Flue-curing of bright tobacco in the R&D curing chamber was continued this month. Some modifications were made to the chamber to meet our needs. A hoist was installed to facilitate the loading and unloading of tobacco.

Three curings were completed in the month of August. The microprocessor and all the controls are functioning properly. Cured leaves were compared with those cured by a farmer, and necessary changes to curing profiles was discussed to gain experience in how to flue-cure bright tobacco in the R&D chamber.

Evolved gas from different stages of curing was collected by C. Kroustalis to determine the compounds volatilized during different stages of curing.

Ethogen treated green tobacco leaves were dried in the semi-works, and will be processed shortly to determine the possibility of pretreating green tobacco leaves for direct utilization of tobacco.

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CHARGE NUMBER: Project 1902

PROJECT TITLE: Microbial Technology

PERIOD COVERED: August 1-31, 1984

PROJECT LEADER: E.D. Mooz

DATE OF REPORT: September 5, 1984

## I. Biotransformation:

Several yeast cultures were rescreened to test for their ability to biotransform ionone. The yeasts were evaluated in three different media and under conditions to increase the rate of biotransformation. Some yeasts do appear to biotransform ionone.

Five Aspergillus cultures previously typed by American Type Culture Collection (ATCC) were examined by scanning electron microscopy. These were compared with a reference culture of Aspergillus carbonarius also obtained from ATCC. The cultures were also examined by light microscopy. The reference Aspergillus carbonarius has a spore size of 9.3 - 12.4mμ, while the other Aspergillus cultures have a spore size of 2.5 - 3.7mμ. Twelve streptomyces cultures also typed by ATCC are being examined by SEM to study their spore morphology.

## II. Recombinant DNA

Studies continue with the screening of plaques for the presence of tobacco actin gene(s). The plaques are transferred to cellulose nitrate filters and hybridized with a $^{32}P$-labeled soybean actin gene.

Work continues on developing methods for DNA sequencing.

D.L. Simpson, Notebook 7585
V.S. Malik, Notebook 8106
J.L. Lyle, Notebook 8038
E. Crockett, Notebook 8041
E.D. Mooz, Notebook 7984

Elizabeth D. Mooz

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Source: https://www.industrydocuments.ucsf.edu/docs/yrfm0000

CHARGE NUMBER: Project 1904

PROJECT TITLE: Bioengineering Studies

PERIOD COVERED: August 1-31, 1984

PROJECT LEADER: I.L. Uydess

DATE OF REPORT: August 30, 1984

Data on the rate and extent of biomass production, sugar and nitrate utilization, and on the accumulation of extracellular (soluble) proteins[1] and pectins are being analyzed and correlated with other analytical data derived from tobacco suspension cultures grown at 27°C (+/-1°C) in either total darkness or total light, and in total darkness at 34°C. While growth rates and production of biomass under each culture condition were generally similar, the degree and rate of utilization of Nitrate[2] and production of extracellular pectins were not. Tobacco suspension cultures acclimated to growth at 27°C in light (approximately 25$\mu$ mol/$m^2$/sec) 24 hours/day utilized nitrate to the greatest degree and produced more extracellular pectins (per gram of cellular biomass) then either of the other two cultures. A special report on these data, as well as on analytical data from extracellular pectin analysis, and analytical investigations of the cellular biomass produced under each environmental situation, are currently being prepared.

Silvia Tenhet and Debbie Chadick participated in a two-week lecture/workshop on plant cell and tissue culture at the University of Kentucky at Knoxville, headed by Dr. Don Dougall. A trip report was issued, summarizing the topics covered.

Dr. Peter Brodelius, an innovator in the field of Plant Cell Immobilization and Permeabilization (to recover intracellular products) from the Swiss Federal Institute for Biotechnology (ETH-Honggerberg) visited PM, R&D on August 19 and 20th. He presented a seminar on his recent work and visited several R&D labs.

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[1]Coomassie Brilliant Blue - reactive soluble proteins.

[2]In relation or response to the availability and utilization of ammonia.

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Source: https://www.industrydocuments.ucsf.edu/docs/yrfm0000

PROJECT CHARGE: 2106

PROJECT TITLE: APPLIED TECHNOLOGY

PROJECT LEADER: J. L. Banyasz

PERIOD COVERED: August 1 - 31, 1984

DATE OF REPORT: August 28, 1984

## AMMONIATION/HEAT TREATMENT OF TOBACCO

In collaboration with Blend Development, the Product Evaluation Division and Flavor Development, we have been attempting to establish a quantitative basis for subjective response to ammonia and heat treated tobaccos. This involved the creation of a "Burley Questionnaire" and the assembly of a volunteer smoking panel. Results to date have established the response to burley and indicate that the procedure does not resolve different stalk positions of burley.

Work is now in progress to establish the responses to bright and oriental tobaccos with respect to the burley characteristics. If these are suitably resolved from burley, then the procedure will be applied to measure the "movement" of treated bright samples toward burley.

## CIGARETTE PAPER - COMBUSTION PROPERTIES

The thermal conductivity tester has been completed and several types of cigarette paper have been examined. The samples included standard production paper, high purity α cellulose and nonporous, low sidestream papers with high inorganic contents. The preliminary data indicate that the thermal conductivities of these materials range from 1.4 to 2 x $10^{-3}$ Watts/cm°C. To date, it appears that additives have relatively little influence on the thermal conductivity of paper. Thus heat conduction appears to play a minor role in the differing burn characteristics of these papers.

## LASER APPLICATIONS

The front optics in the Apollo laser were found to have deteriorated resulting in poor mode. Consequently, repairs were effected. A demonstration of cigarette rod slitting was given for QA and Engineering. Further work is planned in this area now that the laser is functioning properly.

## ASSISTANCE TO OTHER GROUPS

Assistance was provided to Product Development in overhauling the Coherent 525 laser.

J. L. Banyasz

/ev

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CHARGE NUMBER: 2500

PROJECT TITLE: Fundamental Chemistry

PROJECT LEADER: Y. Houminer

PERIOD COVERED: July - August, 1984

DATE OF REPORT: August 30, 1984

The structure-activity program has received much attention in the past two months with major effort devoted to organizing and coordinating the programs. An additional 50 compounds have been profiled and added to the odor data base. In addition to odorants of general interest, several groups of odorants having similar structures were also evaluated. Thus, 2-, 3- and 4-acylated pyridines were studied in order to understand structural parameters related to popcorn-nutty character. A series of low molecular weight aliphatic acids was studied in an attempt to find out where in the series the trigeminal component of the response disappears and the response becomes purely olfactory. Three other series of compounds, namely, courmarin-like odorants, 1,3-dialkylbenzenes and 2,6-dialkylpyridines were also studied recently[1].

We are continuing our work on terpene esters of pyromellitic acid as resolving agents for alkaloids. Treatment of (R,S)-nicotine with p-di-l-menthyl pyromellitate gave the corresponding R- and S-salts. From the R-salt,(R)-nicotine was obtained in 97.6% e.e., and (S)-nicotine was obtained from the corresponding S-salt in 98.9% e.e. A parallel experiment with the diester derived from d-menthol gave similar results.[2] Other diesters of pyromellitic acid were prepared. The l-bornyl diester was reacted with (R,S)-nicotine to give a solid which after a second crystallization was found to yield (S)-nicotine in 95.1% e.e. The (R)-nicotine which was isolated from the mother liquours and had only 78.3% e.e., was crystallized with p-di-l-menthyl pyromellitate to give a diastereomer free of the S-salt. The use of two resolving agents eliminates the need for manual separation of crystals as was required in the case of the l-menthyl derivative. The use of the diester derived from l-isopinocampheol for resolving (R,S)-nicotine is in progress.[2]

Attempt was made to apply the above new resolving agents to other nicotine derivatives. Thus, the l-bornyl derived diester was reacted with (R,S)-5-methylnicotine, (R,S)-6-methylnicotine and (R,S)-nornicotine. This work is currently in progress.[2]

The synthesis of several nicotine analogues has been completed. Thus, 5,6-dimethylnicotine and 6-ethyl-5-methylnicotine were prepared (about 3g of each).

We have renewed our interest in the reaction of some alkylpyrazine anions that can lead to some novel pyrrolopyrazines. Thus, tetramethylpyrazine anion was reacted with pyruvaldehyde dimethylacetal to give the expected alcohol. Experiments to hydrolyze or reduce the acetal function are in progress.[2]

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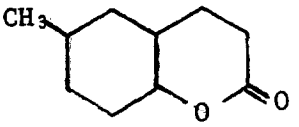
The synthesis of 6-methyl-octahydrocoumarine has been completed. The separation of isomers is in progress.[3]




The strongly fluorescent terbium dipivaloylmethanide was prepared to try to monitor the hydrolytic behaviour of the gadolinium chelate, NaGdEDTA, when applied to tobacco. This research is done in collaboration with Project 2501.

References:

1) Rett Southwick, NB #7798
2) John Paine, NB #8128
3) Mark Sito, NB #8124

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Source: https://www.industrydocuments.ucsf.edu/docs/yrfm0000

CHARGE NUMBER: 2505

PROJECT TITLE: Identification, Characterization and Synthesis

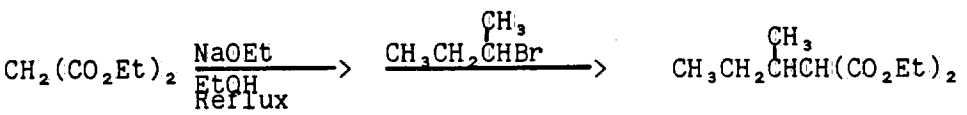
PROJECT LEADER: Roger A. Comes

PERIOD COVERED: July-August, 1984

DATE OF REPORT: August 24, 1984

1. Acid release

In anticipation of future needs and projected large scale requirements in the Oriental replacement program, several 0.2mole scale reactions to prepare diethyl sec. butylmalonate were performed as follows:



The diester is the precursor to the diacid (CR-2403) and its disodium salt (CR-2409) both of which generate β-methyl valeric acid under normal smoking conditions.

Production quantities of these materials should be readily obtainable through a contract vendor. Laboratory scale reactions have generated an inventory of 1.5kg of CR-2409. A procedure for quantitation of β-methyl valeric acid in smoke has been developed by Project Number 1740.

2. GC/MS Support

Multiple (15) samples were analyzed by GC/MS in support of projects within the Chemical Research Division. Additionally, two samples related to a customer complaint were run as requested. A mixture of tobacco alkaloids (approx. 5ng each) was used to evaluate the new "heart cutting" gas chromatograph with excellent results obtained. Modifications are in progress to allow pyrolyses/GC and then attachment to the Finnigan mass spec. will finalize the installation.

3. Organic Synthesis

a) A sample of N'-nitroso nornicotine was prepared at the request of Project 6908.

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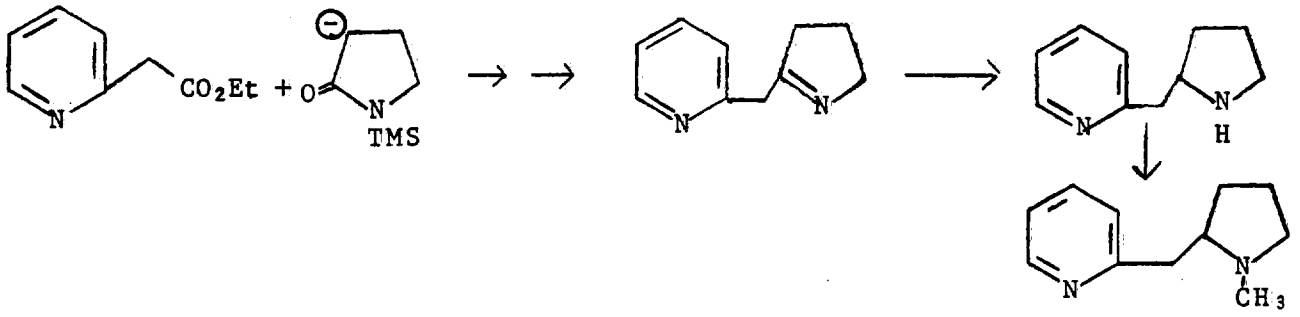
b) A new nicotine analog was prepared as shown:




Interest in this compound comes from the N,N' distance and similarity to nicotine with three carbon atoms intervening between the two nitrogen atoms.

c) Additional experimental work was carried out on the homo and bis homo nicotine analogs for inclusion in proposed publications.

4. Computer support

a) Additional research has been conducted into a laboratory computer system for the Chemical Research Division.

b) Miscellaneous computer applications were addressed. These included: establishment of an inventory file of all capillary GC columns on hand in the division; assistance to the division secretary and divisional personnel in computer familiarization and word processing capabilities; and connection of a liquid scintillation counter to the Perkin-Elmer 3600 computer.

5. Reports

Three special reports issued during this period.

a) "Determination of the Decomposition of a Sucrose Mono-Ester of 3-Methylvaleric Acid in Cigarettes" by D. Ingraham, July 20, 1984, Acc.#84-228.

b) "A Review of the Flavor Release Technology Developed at Philip Morris" by K. Podraza, July 27, 1984, Acc.#84-231.

c) "Data Processing of Signals from Radiochromatography Flow Proportional Counters - Part 2" by D. Ingraham, July 27, 1984, Acc. #84-232.

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CHARGE NUMBER: 2525

PROJECT TITLE: Natural Products Chemistry & Botanical Invest.

PROJECT LEADER: H. J. Grubbs

PERIOD COVERED: July 1 - August 24, 1984

DATE OF REPORT: August 29, 1984

Cis-abienol (12 Z-labda-12,14-diene-8α-ol) is a labdanoid diterpene occurring in certain tobacco[1,2] varieties and is of interest in regard to tobacco aroma and flavor.[3] Clary sage (Salvia sclarea) has been reported to be a rich source of sclareol (labda-14-ene-8α,13-diol). The presence of the diol suggested clary sage as a potential source of cis-abienol. Using HPLC and TLC techniques[4] the presence of large quantities of sclareol was noted. No detectable quantities of abienol were found.

Harvest has been completed in a field study of cis-abienol production in tobacco. In a collaborative effort with the Leaf Department and North Carolina State University, field plot studies are being carried out at the Border Belt Tobacco Research Station, Whiteville, North Carolina. The major objectives in this study are to determine how cis-abienol concentration changes during growth and curing in different tobacco genotypes and what changes take place in other leaf constituents which accompany abienol changes. Six different tobacco genotypes are involved and three different curing methods will be utilized. Leaf disc samples were obtained on a weekly basis starting six weeks after transplant and continuing up to harvest. Cis-abienol as well as other leaf surface components are currently being analyzed by GC and HPLC. Various curing methods (sun-curing, flue-curing, and air-curing) are currently in progress.

In a collaborative effort with Project 1901,[5] a total of 3700 individual tobacco plants were visually screened and selections were made that represent diverse genetic material. These selections were made for further studies in the CGI programs.

Collaboration continues with the Leaf Department and the New Variety Introduction Committee.[6] The Flue Cured Tobacco Variety Evaluation Tours were completed for the Virginia-North Carolina Middle and Old Belt areas, and the Border and Eastern Belt areas. The Burley Tobacco Variety Evaluation Tours were completed for the Virginia-Tennessee cooperative farm plots.

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References:

1. A. Colledge, W.W. Reid and R.D.Russell (1974) Ann Tob. Sect. 2, (11), 159-64.

2. V. Heemann, V. Brummer, C. Paulsen and F. Selhofer (1983) Phytochem. 22, 133-135.

Source: https://www.industrydocuments.ucsf.edu/docs/yrfm0000

3. C. R. Enzell and I. Wahlberg (1980) Rec. Adv. Tob. Sci. 6, 65.

4. S.B. Hassam

5. R. Bass, G. Newell, G. West

6. R. Bass

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CHARGE NUMBER: 1101

PROGRAM TITLE: ENTOMOLOGICAL RESEARCH

PERIOD COVERED: AUGUST 1 - 31, 1984

PROJECT LEADER: D. L. FAUSTINI

DATE OF REPORT: SEPTEMBER 5, 1984

WRITTEN BY: R. M. LEHMAN

## I. CIGARETTE BEETLE (CB) PHYSIOLOGICAL STUDIES

### FIELD

The sex pheromone traps at MC captured 1784 CB while the black light traps caught 879 CB during the month of August. These numbers are up slightly from 1750 and 693 caught in July (1,2).

Culturing processes continue with the wild strain of the CB captured in the Maury Street warehouse. First generation CB from the initial cultures are ready to emerge. Bioassays are being established for determining resistance to DDVP and phosphine (3).

### LAB

Dr. Peter Martin has submitted new cigarette packages wrapped with experimental overwraps and also some packages which have been wrapped following modifications on the packing machines. Hopefully these packages will show improvement as barriers to infestation compared to the first test. Expansion of this test will also allow us to determine more thoroughly the barrier effects of the box versus the soft pack (4).

Separate tests show that packages exposed to adults are infested when eggs are laid at the overwrap seam. The eggs hatch and larvae crawl to the cigarettes through openings along the seam (5).

## II. ASSISTANCE TO OTHERS

Moisture retention/loss is being investigated in the perforated polyethylene bags under actual export conditions. One hundred and twenty-six cases of export cut filler were filled using perforated and non perforated polyethylene bags. Samples from each of the bags were submitted for moisture determinations. Thirty six cases each will be sent to Panama and Switzerland for 6 months storage after which they will be returned to Richmond, analyzed, and compared for moisture changes (7).

2001115293

Source: https://www.industrydocuments.ucsf.edu/docs/yrfm0000

## III. REFERENCES

1. Drew, S. Cigarette Beetle Sex Pheromone Trap Counts; August 6, 13, 20, 27, 1984.

2. Millis, Sue. Manufacturing Center Weekly Bug Count Report for W/E; 8/27/84.

3. Drew, S. Notebook No. 7850, p. 96.

4. Faustini, D. L. Notebook No. 7746, pp. 127-129.

5. Lehman, R. M. Notebook No. 8028, p. 31.

6. Minor, M. F. Notebook No. 7197, p. 156.

7. Long, J. S. Notebook No. 7777, p. 106.

Robert M. Lehman

2001115294

Source: https://www.industrydocuments.ucsf.edu/docs/yrfm0000

CHARGE NUMBER: 1620

PROGRAM TITLE: ELECTROPHYSIOLOGICAL STUDIES

PERIOD COVERED: AUGUST 1 - 31, 1984

PROJECT LEADER: F. P. GULLOTTA

DATE OF REPORT: AUGUST 30, 1984

WRITTEN BY: C. S. HAYES

# I. EVOKED POTENTIALS TO CHEMICAL STIMULI

## A. Nasal Evoked Potentials: Difference Testing Employing Cognitive EPs

Experiments employing cognitive EPs to nasally presented stimuli continue. The stimulus pairs currently being tested are menthol and $CO_2$, and menthol and methyl salicylate (1).

The preliminary data suggest that when these stimulus pairs are compared at different probabilities of occurrence (rare vs. common), a late positive component (LPC) having a latency of approximately 600 - 900 msec. emerges to the rarer stimulus. The LPC is an endogenous, cognitive component reflecting stimulus information processing and is believed to be related to resolution of uncertainty (2).

It has been reported that the degree of confidence subjects have in discriminating differences between stimulus pairs is related to the size of the LPC (i.e., being larger the more confident, or less difficult, the discrimination) (3). The cognitive EPs recorded in our laboratory suggest similar findings. That is, the LPC elicited when comparing menthol and $CO_2$ appears to be larger than when comparing menthol and methyl salicylate, suggesting that the perceived differences between $CO_2$ and menthol are greater than those between menthol and methyl salicylate. Testing in more subjects and among more compounds will be necessary in order to further substantiate these findings and to determine the limits of discriminability that can be obtained employing the technique.

## B. New Olfactometer

A meeting was held this month to discuss the finalized design specifications for the new olfactometer. Plans are for the new olfactometer to be completed by the end of the year.

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## II. THE EFFECTS OF $CO_2$ ON CIGARETTE SMOKE FLAVOR

The retesting of a number of the subjects who appeared to detect the addition of very low $CO_2$ concentrations is currently in progress (4). Also, training in $CO_2$ detection is continuing for a subset of the subjects who participated in the initial $CO_2$ study. Upon completion of training, these subjects will be retested in order to evaluate the effect of prior discrimination training on $CO_2$ detectability.

## III. REFERENCES

1. Hayes, C. S. Notebook No. 8081, pp. 96-119, 125-186.

2. Donchin, E., "Event-related brain potentials: A tool in the study of human information processing." IN: Begleiter, H., ed., Evoked Brain Potentials and Behavior. New York: Plenum; 1979: 13-88.

3. Andreassi, J. L., "Psychophysiology: Human Behavior and Physiological Response." New York: Oxford University Press; 1980: 130-143.

4. Hayes, C. S. Notebook No. 8081, pp. 82, 187.

C. S. Hayes

2001115296

Source: https://www.industrydocuments.ucsf.edu/docs/yrfm0000

PROJECT NUMBER: 1740
PROJECT TITLE : Flavor Components of Tobacco and Filler
PROJECT LEADER: C. S. Kroustalis
PERIOD COVERED: August, 1984

## I. FLAVORS

A. The major glycoside peak from fraction HF-15' was trapped using a 50cm x 9.4mm reversed phase Magnum HPLC column. FAB/MS (Dr. Einolf) gave the glycoside, aglycone and glucose molecular weights. Enzymatic hydrolysis of the glycoside yielded the aglycone which was identified by GC/MS as 3-oxo-α-ionol.

B. Cigarettes treated with sec-butylmalonic acid and disodium sec-butylmalonate were analyzed for percent transfer of β-methylvaleric acid to mainstream TPM. Percent transfer was ≈3% for both compounds. β-methylvaleric acid deliveries were 41 and 28 µg/cigarette from available calculated β-methylvaleric acid concentrations of 1285 and 890 µg/cigarette, respectively. A report is in preparation.

C. A series of block chocolate samples from several suppliers are currently analyzed to determine the concentration of theobromine. This is in response to low theobromine concentration in block chocolate used by Manufacturing.

D. Two CF substitute flavors were analyzed for Operations Services to determine whether CF was present. No CF was found.

E. Two full experiments (~100 samples) of biotransformed ionones were profiled during this month.

## II. CURING STUDIES

Two flue-curing cycles from Dr. Teng's curing "Barn" have been sampled for headspace volatiles and one for steam distillates and cuticular components. Headspace samples were generally low in volatiles. Estimated total volatiles from ~60 pounds of cured weight bright tobacco were 300-400 µg/cubic meter of headspace. During the early stages of curing (2-3 days), only volatile components were present. As the "barn" temperature increased above 45°C, the volatile compounds decreased while nicotine and higher boiling compounds increased in concentration. Steam distillates also showed changing patterns during the curing cycle. Of the cuticular compounds only the hydrocarbons ($C_{25}$-$C_{33}$) remained relatively unchanged. Alcohols ($C_{18}$-$C_{22}$) appear to increase for a time during the cycle followed by a decrease in concentration in the cured tobacco. α&β-4,8,13-duvatriene-1,3-diol changes were similar to straight chain alcohols except that their concentration decreased drastically in the cured sample. Steam distillates and headspace samples will be submitted for GC/MS identification.

## III. OTHERS

A. Volatile fatty acids were determined in RL samples with an off-taste to pinpoint the RL production date of the hogsheads in question. Volatile

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fatty acids were also determined in samples from Dr. Teng's aging studies.

B. Rutin and chlorogenic acid were determined in Ecuadorian black tobacco competitive cigarettes. Rutin and chlorogenic acid were used as indicators of bright tobacco in the blend.

C. Assistance was provided in sampling and ion chromatography chloride determination from the CGI North Carolina field.

D. Residual ethanol was determined in foil-applied menthol and control cigarettes.

E. Several customer complaint cigarettes were analyzed. Two major contaminants were present: 1) Merit cigarettes from two different locations contained isomers of dimethyl or ethyl phenylmethyl benzene (identified by J. Naworal); 2) Merit and B&H Ultralights were contaminated with tax stamp ink solvents.

## IV. MEMO

C. S. Kroustalis to R. Carpenter, "Marlboro K. S. (CC #27-84)", August 28, 1984.

C. S. Kroustalis

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PROJECT NUMBER: 1750
PROJECT TITLE : Pyrolysis and Combustion of Tobacco
PROJECT LEADER: John O. Lephardt
PERIOD COVERED: August, 1984

## I. FT-IR-EGA STUDIES

### A. Tobacco Seeds

Five varieties of tobacco seeds have been examined to determine whether FT-IR-EGA could discriminate between tobacco materials at this stage. From the preliminary results, some small differences were noted for all types examined. Seedlings soon after germination will also be examined as part of an effort to explore FT-IR-EGA as an early diagnostic tool.

### B. Glucosylamine and Rhamnosylamine

EGA profiles of these materials are quite similar with the principal exception of a presently unknown component that evolves from the rhamnosylamine. This component appears to derive from the rhamnose sugar and additional analyses to define it will be performed.

### C. Supercritical $CO_2$ Extracted Tobacco Material

For inclusion in our base collection of profiles for tobacco materials, uncased bright, burley and oriental tobaccos which had been extracted with supercritical $CO_2$ were examined. The principal features observed for bright tobacco were reductions in sugars and nicotine. Using evolutions in the higher carbohydrate regions (which presumably would not be significantly affected by the treatment), the weight loss due to the extraction process was estimated at 8%.

## II. METHODS DEVELOPMENT

### A. Spectromicroscopy

An analytical technique that may overcome a long held contention that one cannot resolve spectral features below the diffraction limit of the radiation involved has been proposed. The technique would utilize modulated radiation and synchronous detection of modulated components in an electron microscope signal channel. The requirements and alternate mechanisms for testing this technique are being examined.

### B. Hardware/Software Development

1. Revisions to some of our software routines on both the Digilab FTS-20E minicomputer system and the DEC 2060 system were made to allow better manipulation and plotting of spectra using the DEC 2060.

2. The pressure formatter system was received, but a problem with the file size and communications system must be resolved before this system will be operational.

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3. A special thanks is extended to Brian Jeltema for developing a better communications software system between our Digilab FTS-20E spectrometer and the DEC 2060. This system improves both the speed and reliability of communications between these machines.

J. Leyhandt

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PROJECT NUMBER: 1752
PROJECT TITLE : Cigarette Smoke Studies
PROJECT LEADER: Gunars Vilcins
PERIOD COVERED: August, 1984

## I. AMMONIA THRESHOLD STUDY

The equipment for the collection and testing of cigarette smoke with added known amounts of ammonia has been designed and interfaced for the ammonia threshold study. A procedure for determining the threshold level has been developed by the joint efforts of members from Flavor Development, Product Evaluation, and Analytical Research Divisions. The testing to determine the concentration threshold curve has begun using E-55 Bright tobacco cigarettes. Six out of six panelists identified correctly the test smoke sample with added ammonia at a concentration level of ~600ng/cc on a triangle test.

The study is continuing.

## II. AMMONIA - AMMONIUM IN CIGARETTE SMOKE

Six different types of cigarettes were selected and tested for $NH_3$ in cigarette smoke. These cigarettes will be used in the development of a procedure for the determination of ammonia plus ammonium ($NH_3 + NH_4^+$) in cigarette smoke using the ion chromatographic procedure. This phase of work is proceeding with the cooperation of Bill Harvey, Dick Davis, and Glenn Baker from Project #6505.

## III. SIDESTREAM SMOKE

Investigations have been made to develop a procedure for the quantitative determination of major gaseous components of cigarette sidestream smoke. At this time, several smoke samples have been collected by Barbu Demian, Project #4009, and analyzed using the Nicolet 160 FTIR spectrometer. It appears that carbon monoxide, carbon dioxide, and ammonia can be determined quantitatively in sidestream smoke using this procedure. However, possible problem areas, such as aging effects, have not been investigated.

## IV. MEMOS

A. Milton Parrish to Richard Cox, "TDL $NH_3$ Deliveries for Cigarettes with Glucosylamine and 5% Rhamnosylamine", August 7, 1984.
B. Gunars Vilcins to Barbro Goodman, "Retesting of Passport Cigarettes", August 8, 1984.

G. Vilcins

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PROJECT NUMBER: 1754
PROJECT TITLE : Spectroscopic Studies of Tobacco and Smoke Components
PROJECT LEADER: W. N. Einolf
PERIOD COVERED: August, 1984

## NMR

Two 2,4-disubstituted butyrolactones were analyzed for Ken Podraza to determine the cis/trans configuration by using chemical shift and coupling constant data. A kinetic study of the rhamnose-ammonia reaction showed that the reaction is 75-80% complete after 18 hours and complete after 72 hours (no rhamnose detected). Several serricornin and anhydroserricornin samples were analzyed to determine isomer ratios for the diastereoisomers.[1]

The carbonyl/carboxyl region of the $^{13}C$ CP/MAS spectra of tobacco is very distinctive for different tobacco samples. The spectra of several model compounds were obtained, including citric acid trisodium salt dihydrate, citric acid monosodium salt (anhydrous), and malic acid disodium salt dihydrate. The sharp 180 ppm resonance in burley tobacco may be due to the monosodium (or some other cation) salt of citric acid since it gives a sharp resonance at 180 ppm and a weaker signal at 173 (which would overlap with the uronic acid carboxyls in tobacco spectra). The free citric acid or the trisodium salt gives three resolved signals which do not resemble the tobacco signals. The malic acid salt seems to be ruled out since the carboxyl signals are shifted slightly downfield to 182 & 183 ppm. The two malic carboxyl signals were found to have different cross polarization efficiencies and rotating reference frame relaxation times.[5]

$^{13}C$ CP/MAS spectra were also obtained on pansy flowers, a whole freeze-dried tobacco cell suspension culture, and several carbonate salts.[5]

## MS

A new 160 M byte disk was installed on the SS 200 data system and working files were transferred to it. A head crash occurred on the 30 M byte disk, causing a loss of our libraries. We were able to recover about 70% of the data, and we are in the process of rebuilding the libraries. Additional problems with our magnetic tape unit has also caused a slow down in recovery from these data system problems.[3]

Four rhamnosylamine samples were analyzed for G. Chan using DCI/MS. Each was found to be pure.[2]

Several compounds were evaluated for use as FAB calibration standards. A perfluoro compound, Ultramark 2500, was found to be excellent for negative FAB, since its mass spectrum contains abundant ions at masses from 50 to 1000 at intervals of about 50u. Additional data have been obtained by FAB for tobacco glycosides and for a tobacco leaf extract fraction isolated by HPLC by Bob Armstrong. In general, the glycosides show strong M + 1 ions, and ions corresponding to loss of the saccharide units. They also readily form sodium and potassium adduct ions in the positive mode.[2]

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## ELECTRONICS[4]

A problem in a printed circuit board was identified and corrected on the Nermag MS, thus avoiding a downtime of several weeks. The gas cell on the P. E. 221 IR was overhauled and realigned, thus improving signal transmission from 30% to 55%. Repairs were made on other various IR instruments and a digital voltmeter was constructed and installed on the MAT 112S MS to provide a digital mass display.

A considerable amount of time was spent in efforts to correct computer problems on the Bruker NMR. Replacement printed circuit boards have been ordered.

## REFERENCES


1. R. Bassfield, N.B. 7398.
2. W. N. Einolf, N.B. 8040.
3. D. F. Magin, N.B. 7936.
4. S. C. Marrs
5. J. B. Wooten, N.B. 7826.

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PROJECT NUMBER: 1756
PROJECT TITLE : Analytical Sensory Correlations
PROJECT LEADER: B. W. Good
PERIOD COVERED: August, 1984

## I. SMOKE

**A. Fractionation.** The precision of the standardized fractionation method for TPM was evaluated by repeatedly analyzing Marlboro 85mm cigarettes. The data collected on the HP3357 LAS was transferred to the DEC 20/60 for BMDP analysis. BMDP1D (simple data description) was used to assess the variability of the raw peak areas. The results revealed that acidic and neutral fractions were more reproducible than basic fractions. The areas for major and minor peaks varied between 5 and 70%. Because of the wide MW distribution, internal standards could only correct for part of variability. In order to estimate the variability contributed by smoking, the same method will be applied to analysis of uncased burley tobacco.

**B. Organic Gas Phase.** Hand made Merit, Merit treated with glucosylamine, Marlboro, and Marlboro treated with rhamnosylamine were evaluated for organic gas phase. Based on a sample size of only four cigarettes, there were no significant statistical differences between the test and control cigarettes of both studies. Those that were so designated were within the limits of a normal distribution.

## II. TOBACCO

**Carotenoids.** To aid the identification of tobacco carotenoids by LC/MS, a photodiode-array detector is on loan from HP to acquire some spectral data.

## III. MISCELLANEOUS

**LABSAM.** The primary system of LABSAM is now operational!

## IV. REPORTS, PRESENTATIONS AND MEMOS

Memo to R. H. Cox from B. W. Good and T. D. Crews, "Cigarettes treated with Glucosylamine and Rhamnosylamine", August 28, 1984.

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PROJECT NUMBER: 1758
PROJECT TITLE : Tobacco Cell Wall Research
PROJECT LEADER: Gordon H. Bokelman
PERIOD COVERED: August, 1984

## I. TOBACCO STALK PITH (Howard Sun and Gordon Bokelman)

For the purpose of biological testing, tobacco sheets were prepared from both bright and burley 1983 lyophilized stalk pith. The bright sheet contained 100% stalk pith, but it was necessary to add 2% citrus pectin as a binder to the burley stalk pith.

On August 2, one hundred field-grown burley stalks were defoliated, harvested, cut into ~1 inch sections and frozen at the V.P.I. & S.U. Southern Piedmont Research and Continuing Education Center in Blackstone, VA , for further processing at Philip Morris.

## II. BLEND COMPOSITION ANALYSIS (Howard Sun, Jack Stimler and Gordon Bokelman)

With the new tobacco data base, the following correlation coefficients were found for DBC burley content: free aspartic acid - 0.98 and free glutamic acid - 0.92. The consistency of free aspartic acid as a predictor is being evaluated using different batches of DBC burley.

The fact that the best predictors for burley are low molecular weight acids (citric, aspartic and glutamic) raises the intriguing possibility that burley's unique pharmacognosy may define it as a distinct chemical race.

## III. INSTRUMENTATION (Bill Ryan)

A new Hewlett-Packard 5880 A gas chromatograph was received and installed. This instrument has electronic flow control (EFC) of the split/splitless injection port. With EFC, it is possible to rapidly establish appropriate column head pressures and split ratios. The use of this feature to program column head pressure during a gc run and thus improve the separation by decreasing retention time and/or improving peak shape is being investigated.

## IV. STRUCTURAL STUDY OF TOBACCO PECTIN (Howard Sun)

A pectin isolated from E-55 bright tobacco was converted to a neutral polysaccharide by reduction with sodium borodeuteride in the presence of 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-p-toluenesulfonate. The reduced pectin contained 78.8% galactose, 14.8% rhamnose and 6.4% arabinose as determined by gc quantitation after methanolysis and silylation. The increase in rhamnose and arabinose contents (compared to starting material) clearly indicates that a significant amount of galacturonic acid was degraded during the reduction. However, this neutral polysaccharide should be suitable for determination of the neutral sugar linkage patterns following partial acid hydrolysis.

## V. ACID-DETERGENT RESIDUE DETERMINATIONS (Gordon Bokelman)

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Acid-detergent residue analyses were performed on a number of bright and burley tobacco samples at the request of Betty Handy. This procedure was seen

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to give reproducible results. However, interpretation of the values obtained was more meaningful for freeze-dried rather than cured tobacco samples. This is a reflection of the fact that tobacco proteins undergo complex condensation reactions during curing to form insoluble products.

## VI. MISCELLANEOUS

1. Memo to Laura McCray from Gordon H. Bokelman, "Pectin in Dust Samples from the BL Plant", dated August 13, 1984.
2. Memo to Dr. R. H. Cox from G. H. Bokelman, "1984 Annual Meeting of the American Society of Plant Physiologists", dated September 4, 1984.

Gordon H. Bokelman

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PROJECT NUMBER: 6505
PROJECT TITLE : Special Investigations and Methods Development
PROJECT LEADER: William R. Harvey
PERIOD COVERED: August, 1984

## I. HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

Anticircular chromatography was discontinued because of overloading with nicotine. A return to a linear form of chromatography appears adequate for our needs for alkaloids separation.

## II. AMMONIUM ION IN SMOKE

Ion chromatographic parameters for the determination of ammonium ion in trapped smoke solutions have been determined. There is linearity in the range of 1-5ppm ammonium concentration and ammonium ion can be seen on trapped smoke solutions. A smoking machine has been assembled and calibrated for puff volume and duration for use by Project 1752.

## III. ORGANIC ACIDS IN TOBACCO LEAF

Better agreement with the gas chromatographic procedure for malic acid was achieved by using the normal anion column rather than the ion exclusion column (ICE). Evidently, there is a coeluting compound which interferes in the ICE determination.

## IV. SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC)

Poor precision for the SFC determination of propyl and heptyl parabens was traced to a low slope sensitivity integrator setting. Better precision was realized by hand reading. A visit was made to Dr. Milton Lee's laboratory at Brigham Young University to learn to operate and troubleshoot the capillary SFC being built for us. Problems still exist in bringing the system up.

## V. PHOSPHINE SCRUBBING OF FUMIGANT EXHAUSTS

Efforts are in progress to determine the most practical way of scrubbing phosphine ($PH_3$) before it is released to the environment. Both liquid and solid scrubbers are being considered. Both modes appear to rely on the ultimate conversion of phosphine to phosphate.

William R. Harvey

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CHARGE NUMBER: 6902

PROGRAM TITLE: BIOLOGICAL SPECIAL INVESTIGATIONS

PERIOD COVERED: AUGUST 1 - 31, 1984

PROJECT LEADER: A. H. WARFIELD

DATE OF REPORT: SEPTEMBER 7, 1984

## I. TSNA IN FILLER: MODEL STUDIES (1,2,3)

Further investigations were conducted this month on the problem of TSNA formation during air curing of tobacco filler. As described in the July report, a modified homogenized leaf curing system was used as a model.

An aqueous slurry was prepared by grinding green, field-grown bright tobacco (4) in a blender. Particulate matter was filtered on a celite bed, and the filtrate was divided into four portions, each representing 4.5g dry weight. Nicotine citrate (50 mg of nicotine) was added to each portion. Sodium nitrate (15 mg $NO_3^-$-N) was added to two portions, and potassium nitrite (15 mg $NO_2^-$-N) to the remaining two portions. One of each of the nitrate and nitrite-enriched portions was sterilized by 0.2μ filtration, after which all four samples were statically incubated at 37°C for 24 hr followed by 20°C for 4 days. No substantial changes in nicotine content were observed. In the samples containing added nitrate, the presence of bacteria caused nitrate to be reduced to nitrite, and fairly high levels of TSNA were found. The sterile samples containing added nitrate did not undergo any changes in nitrate or nitrite, and only insignificant levels of TSNA were found. In the samples where nitrite was added, high levels of TSNA were found, regardless of whether bacteria were present, and nitrate/nitrite ratios did not change. It was concluded that the major role of bacteria is the reduction of nitrate to nitrite, and that the formation of TSNA occurs via chemical nitrosation of either the nicotine or secondary amine minor alkaloids.

The relative yields of TSNA from nicotine, nornicotine, anatabine and myosmine were determined by treatment of the alkaloids with nitrite in pH 5.5 citrate-phosphate buffer for 23 hours at 37.5°C. The results are shown below:

| | % Yield | | | |
|---|---|---|---|---|
| | NNN | NNK | NNA | NAT |
| nicotine | 0.007 | 0.017 | 0.16 | 0.019 |
| nornicotine (5) | 1.7 | ND | ND | ND |
| anatabine | ND | ND | ND | 8.4 |
| myosmine (5) | ND | ND | ND | ND |

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NNA and NNK are ring-opened compounds derived from nicotine. NNA is not isolated in the tobacco matrix, but has been observed in low temperature reactions previously (6). Since the yield of NNN from nicotine was only 1/250 that from nornicotine, the latter alkaloid is probably an important precursor of NNN in filler. The NNN/NNK ratio from nicotine was 1:2.4, as opposed to 15:1 from the bright tobacco model. This could also be due in part to the presence of nornicotine in tobacco, or to other factors such as pH, temperature, metal ions, etc. An attempt was made to find nornicotine in the base fraction from the initial filtrate from green burley, but none could be found (7). Further attempts will be made.

In another experiment using green field-grown bright tobacco, the effect of incomplete filtration or no filtration was determined. It was found that TSNA are produced if all the insoluble material is removed, but the presence of a green, colloidal suspension essentially eliminates TSNA production, and causes all nitrate to be removed without buildup of nitrite. When the filtrate was incubated at 37.5°C for 3 days, NNK could not be detected, but at 21°C/6 days the NNK was 2X the NNN. It was also shown that one organism, isolated in pure culture from the previous sample of bright tobacco, was capable of extensive reduction of nitrate to nitrite and production of TSNA when inoculated into a sterile filtrate and incubated at 37.5° for 3 days, while another organism isolated from the same sample was much less effective despite prolific growth in the model system.

Further experiments are being planned to study the effects of temperature, pH, inhibitors, etc., on TSNA production in these systems. The role and mechanism of action of the green collodial material will also be examined, as will the possible effect of metal ions.

## II. REFERENCES

1. Warfield, A. N. Notebook 8063.
2. Yu, T. Notebook No. 7729.
3. Petri, D. L. Notebook No. 7701.
4. Obtained from D. Teng.
5. Obtained from H. Secor.
6. Hecht, S. S.; Chen, C. B.; Ornaf, R. M.; Jacobs, E.; Adams, J. D.; Hoffmann, D. Reaction of nicotine and sodium nitrite: formation of nitrosamines and fragmentation of the pyrrolidine ring. J. Org. Chem. 43, 72-76, 1978.
7. Kinser, R. Notebook No. 8137.

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A. N. Warfield

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CHARGE NUMBER: 6904

PROGRAM TITLE: BIOCHEMICAL METHODS DEVELOPMENT AND UTILIZATION

PERIOD COVERED: AUGUST 1 - 31, 1984

PROJECT LEADER: R. W. MCCUEN

DATE OF REPORT: SEPTEMBER 4, 1984

## I. V79 INHIBITION OF METABOLIC COOPERATION (IMC) ASSAY

With the expert assistance of Mr. John Tindall of the Product Evaluation Division, a method was developed for determining specific activity (percent recovery of 6-thioguanine resistant cells per ug/ml of test substance) (1). The IMC data best fit various types of a linear mathematical model so that the slope is an accurate representation of specific activity. Table 1 gives the specific activity of a variety of agents tested in the IMC assay. The larger the number the more active the agent.

Table 1. Specific Activity of Several Agents Tested in the IMC Assay.

| TEST AGENT | SLOPE (Specific Activity)* |
|---|---|
| 12-O-tetradecanoyl-phorbol-13-acetate | 46443 |
| catechol | 11 |
| CSC from LTF-5E | 2 |
| CSC from 2R1 | 0.8 |
| saccharin | 0.002 |
| phenol | not active |

* Specific activity = percent recovery of 6-thioguanine resistant cells per ug/ml of test substance.

Mr. Tindall also described a statistical method for comparing the specific activities of two active agents to determine if they are differentially active. When this method (analysis of variance followed by separation of means) was applied to the agents listed in Table 1, each was separable from the other (differentially

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active) at the 95% confidence level. These statistical techniques will be applied to other test substances when the data are available, keeping in mind that specific activity should also be determined using other units of measure (nanomoles, cigarette deliveries, etc.).

Laboratory work has been completed with cigarettes containing high and low alkaloid filler levels (2.75% versus 0.51%) (2). While these CSCs were active, they did not appear to be different from each other. Confirmation of this conclusion as well as the relationship of these CSCs to the agents given in Table 1 awaits the analysis of the data using the statistical techniques described above. These latter two statements were also true for the cigarette with high nitrate in the filler (0.91%) collected by two different condensate trapping methods (dry, impaction-trapped processed versus cold-trapped processed) (3). All of this effort is directed towards attempting to understand what controls cigarette activity in this assay. CSCs from a 100% bright (2), a 100% burley (2) or a synthetic, nitrogen-free (LTF-2A) (3) filler are currently being tested to help answer this question.

Data have been generated and statistically analyzed which revealed that CSC obtained from synthetic, nitrogen-free filler (LTF-5E) can be stored (-80°C in dimethyl sulfoxide) for at least 2 months without losing any significant IMC activity (3).

Incorporation of an exogenous metabolic activation system with this bioassay has been hampered by the lack of success with obtaining a cofactor mix (as part of the metabolic activation system) that is compatible with the assay. We have determined that it is the energy source in the cofactor mix (the NADP or NAPDH) that in some way(s) renders the 6-thioguanine sensitive cells immune to killing by 6-thioguanine (3,4). This affords the sensitive cells a longer time to metabolically cooperate with resistant cells which finally results in a greater loss of resistant cells in the presence of the cofactor mix than in the absence of the cofactor mix. Currently we are attempting to determine if this "enhancement of metabolic cooperation" will be a problem when actual test agents are used in the presence of cofactor mix. Attempts will be made to alleviate the cofactor mix block by rinsing the plates after the cofactor mix treatment or increasing the concentration of 6-thioguanine in the treatment medium.

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II. REFERENCES

1. McCuen, R. W. Notebook No. 8136; pp. 5-6.
2. Penn, J. M. Notebook No. 8104; pp. 71-72.
3. Ayers, D. J. Notebook No. 8107; pp. 105-109.
4. Garcia, H. D. Notebook No. 8051, p. 167.

R. W. McCuen

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CHARGE NUMBER: 6906

PROGRAM TITLE: BIOLOGICAL EFFECTS OF SMOKE

PERIOD COVERED: AUGUST 1-31, 1984

PROJECT LEADER: C. K. ELLIS

DATE OF REPORT: SEPTEMBER 5, 1984

WRITTEN BY: C. J. HACKETT, JR.

## I. SALMONELLA/MICROSOME (S/M) MUTATION ASSAY: ACTIVITY OF THE PAH III FRACTIONS OBTAINED FROM NORMAL VERSUS LOW ALKALOID IT CSC.

An experiment was conducted using the S/M assay (TA100+S9) to determine the activity of PAH-III fractions from normal versus low alkaloid IT CSC (1). The two samples had similar PAH-III fraction yields (2), but the corresponding CSCs had different estimated biological activities (EBA). The PAH-III fraction was tested in order to determine if S/M activity correlated with the PAH-III yield or the EBA of the corresponding CSC. There was little or no difference in the specific and total activity of the two samples. The total activity (S/M) of the PAH-III fractions per mg of normal and low alkaloid IT CSC appears to correlate with the PAH-III yield data and not the EBA of the corresponding CSC.

## II. S/M ASSAY: DETERMINATION OF THE ACTIVITY OF THE TPM COLLECTED USING VARYING PUFF PARAMETERS.

In order to determine if the way a cigarette is smoked affects biological activity, the S/M assay (TA98+S9) was used to determine the activity of TPM collected from a burley cigarette smoked using the standard puff parameters (2 sec., 35 cc), increased puff volume (2 sec., 70 cc), and shorter puff duration (1 sec., 35 cc) (1). By increasing the puff volume (2 sec, 70 cc), the specific activity (rev/mg) of the resulting TPM was reduced by 20% versus the standard puff TPM. However, the total activity (rev/cigt) of the standard puff TPM was approximately 33% lower than the increased puff volume TPM due to the higher yield of TPM produced by the increased puff volume. The TPM collected from using the shorter puff duration was not significantly different in specific or total activity from the TPM collected using the standard puff parameters.

## III. S/M STRAIN TA102: DETERMINATION OF STRAIN SPECIFICITY

An experiment was performed in order to evaluate if strain TA102 can more readily detect the activity of some compounds than either strain TA98 and TA100 (4). The reversion rates of 2AF,

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$H_2O_2$, HCHO, mitomycin C (MMC), methylmethanesulfonate (MMS), and freshly smoked 2R1 CSC were determined in TA98, TA100, and TA102 with and without metabolic activation. Even though low titers and a low spontaneous reversion rate was observed, the results indicated $H_2O_2$ (-S9), MMC(-S9), MMS(+S9) and MMS(-S9) were more active in TA102 than in TA98 and $H_2O_2$ (-S9), MMC(+S9), and MMC(-S9) were more active in TA102 than in TA100. Therefore, strain TA102 appears to more readily detect the activity of certain compounds. The results of this experiment confirmed previous results indicating that 2R1 CSC is weakly active (+S9) in strain TA102 (5). Plans are to determine the cause of the low titers and reversion rates and to evaluate different model cigarettes and CSC fractions using strain TA102 to determine if the specificity of strain TA102 will be of value to us.

## IV. TISSUE CULTURE: GLUTATHIONE DEPLETION ASSAY (GDA) AND CELL DEATH

Depletion of cellular glutathione levels by 2R1 CSC has been demonstrated (6). To investigate the significance of the depletion, preliminary experiments were conducted to detemine if a correlation exists between glutathione depletion and cell death following exposure of V79 cells to 2R1 CSC. Cells were exposed to concentrations of 0, 250, 500, 1500, and 2500 µg/ml of 2R1 CSC. Immediately following the 30 minute treatment period, the V79 cells were examined and the percent of dead cells (uptake of trypan blue stain) was calculated. The percentage of dead cells per plate ranged from 2% to 9%, and there was no correlation between glutathione depletion and the percent dead cells. Therefore, glutathione depletion does not appear to be an indicator of cell death.

## V. TISSUE CULTURE: EFFECTS OF 2R1 CSC ON V79 CELLS AND GROWTH RATES (7)

V79 cells were treated with 1500 µg/ml CSC for 30 minutes, the CSC was washed off with phosphate buffered saline (PBS) and media was returned to the plate. The total number of cells and the percentage of dead cells on each plate was determined 24 and 48 hours later. There was an increase in the cell population in the first 24 hours and the total number of cells per plate reached a plateau when the cells became confluent. The change in the cell population density during the 48 hour period was indicative of normal cellular growth rates. In addition, there was no evidence of irreversible damage to the cells that was not apparent immediatedly following exposure to 2R1 CSC (30 min). The toxicity of the CSC will be evaluated with another "toxicity" endpoint (the ability of the cells to form clonies) in future experiments.

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VI. THE BIOCHEMICAL EFFECTS OF SMOKE: GLUTATHIONE DEPLETION ASSAY SIX MODEL CIGARETTES (8)

The CSC from six model cigarettes (2R1-Kentucky reference, bright, burley, LTF-5E, RCB, and RL reference) was retested in the GDA. In a previous experiment (9) when PBS (with $Ca^{++}$ and $Mg^{++}$) was utilized, 2R1 CSC produced an increase in the oxidized glutathione (GSSG) levels of V79 cells with increasing CSC doses (10). The increased GSSG levels were not evident when media was utilized during the treatment periods in a subsequent experiment. Since certain media components can cause chromatographic interference and may compete wth GSH by binding certain CSC components, the six models CSC's were evaluated using PBS (with $Ca^{++}$ and $Mg^{++}$). The results of this experiment indicated that GSSG levels did not increase with increasing doses of CSC. A dose response depletion of GSH was evident for all models tested. The LTF-5E CSC caused more GSH depletion than any other model tested. More depletion of GSH was observed when PBS was used during the treatment period than when media was used. This may be due to the competition of other SH compounds (L-cysteine, L-cystine, ascorbic acid) found in the media for the CSC component.

VII. THE BIOLOGICAL EFFECTS OF SMOKE: GLUTATHIONE DEPLETION - A COMPARISON OF LTF-5E AND LTF-2A AND THE DEPLETION OF GSH BY 2R1 CSC AND NICOTINE WITH AND WITHOUT CELLS (11).

Since, to date, LTF-5E CSC is the most active CSC tested in the GDA, an experiment was conducted to compare the activity of LTF-5E CSC (a cigarette with a high organic filler) with that of LTF-2A (a cigarette with a low EBA and a low organic filler). In addition, the depletion of GSH by nicotine and 2R1 CSC in V79 cells was compared to the depletion of GSH standard obtained without cells present. A dose response depletion on GSH (using V79 cells) was evident for all samples tested. The most depletion was seen with LTF-5E, 2R1 CSC was intermediate, and LTF-2A and nicotine showed the least depletion. Standards dosed with 2R1 CSC and nicotine also showed a dose related depletion. Overall, when compared with the depletion of GSH in cells, more GSH depletion was observed with an equivalent amount of 2R1 CSC and nicotine without cells present. These experiments will be repeated; however, these preliminary results indicate that the presence of cells can make a difference in the results observed with the GDA.

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Charles J Hackett, Jr

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CHARGE NUMBER: 6908

PROGRAM TITLE: SMOKE CONDENSATE STUDIES

PERIOD COVERED: AUGUST 1 - 31, 1984

PROJECT LEADER: S. TAFUR

DATE OF REPORT: SEPTEMBER 5, 1984

## I. NITROSAMINES

Experiments using model LTF-5E cigarettes to study the pyrosynthetic formation of tobacco-specific nitrosamines (TSNA) have continued (1,2). TSNA have been detected in low, but varied, levels in all LTF-5E fillers prepared to date with a trend for higher levels of preformed TSNA in LTF fillers with higher nicotine content. Addition of nitrate at different concentrations to the LTF filler with nicotine does not appreciably change the TSNA content (1). Based on transfer studies which showed low transfer rates of preformed TSNA to smoke, all of these LTF fillers with low amounts of preformed TSNA can be used in smoke studies. When nitrite was added to the LTF formulation, considerably higher levels of TSNA were found in the filler with NNK over 1000 ng/g (2).

The possibility of solvents, reagents or glassware contributing to a background level of TSNA in samples was eliminated by appropriate control analyses (1). Analysis of a completely different LTF formulation (LTF-IIA) (3) indicated no detectable TSNA. Other possible sources of TSNA in LTF-5E fillers are being investigated.

As reported previously, TSNA in smoke were detected from an LTF filler containing nicotine, but in the absence of a nitrate precursor (4). When a higher level of nicotine was added to the LTF filler without nitrate, NNK in SS was the only TSNA to increase with the higher nicotine level (2).

The TSNA content of smoke from LTF fillers with ~2% nicotine and varying levels of nitrate (5) was determined (1). Only SS NNK values indicated a dependence on nitrate. Nicotine and nitrate in the LTF at levels comparable to 2R1 filler yielded lower than expected MS TSNA or SS NNN and NAT. These results suggest that pyrosynthesis of NNN and NAT in tobacco may involve other sources of nitrosating species or other factors which influence the reaction(s). Nitrite, added as an alternate nitrosating precursor to the LTF filler with nicotine also gave relatively low MS and SS TSNA levels (2).

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Examination of the use of 2R1F instead of 2R1 cigarettes as the basis of comparison for the LTF studies has continued (2). The TSNA level in MS smoke from 2R1F cigarettes with filters removed is somewhat higher than the level from 2R1 MS. This difference reflects the effect of the butt tobacco in 2R1 cigarette on delivery of TSNA into MS smoke. The net increase in TSNA measured in the butts is much greater than the amount which would normally be retained by butt filtration. Ascorbyl palmitate, an inhibitor of nitrosamine formation, was injected into the butt portion of 2R1 cigarettes to determine if the increased TSNA in butts was due to pyrosynthetic formation (2). The butt TSNA level showed a large reduction with only a slight decrease in MS TSNA. Further work in this area is planned.

## II. PAH

An internal standard (ISTD) method for the GC analysis of PAH III fractions has been established (6). Using biphenyl as the internal standard, two ISTD modes for determining baselines were compared to the default mode without an ISTD for integrating the total area of the PAH III fraction as well as for a single component (pyrene). The integrated area of the discrete peaks of a total PAH III fraction accounts for ~62% of the injected amount of material. The remaining 38% of the injected sample consists of components below the baseline and probably a small amount of non-volatile, undetected material.

## III. MAINSTREAM SMOKE STUDIES

Filter efficiencies were determined for a set of model cigarettes with identical CA filters using impaction-trapped CSC and TPM deliveries (7). The cigarettes included single component fillers (bright, burley, oriental, ES, SS) and one blended filler. Their filter efficiencies ranged from 20 to 34% with no dependence on CSC or TPM delivery. The theoretical contribution of single components to the blended cigarette MS CSC or TPM and the theoretical total delivery were then calculated. Excellent agreement was observed between the theoretical and actual deliveries for both filtered and non-filtered cigarettes (8). Similar calculations were performed using TPM, nicotine and FTC tar deliveries of the same cigarettes smoked by the CI lab. Again, good agreement was found between calculated and actual blend deliveries (7).

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To determine the effect of varied puff volume and puff duration on TPM delivery and S/M activity of the TPM, 100% DBC burley cigarettes were smoked on the SAVA smoking machine (7). The smoking parameters included the normal 2-sec 35cc puff, a 1-sec 35cc puff and a 2-sec 70cc puff. The TPM deliveries from the

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35cc puffs were similar even though the flow velocity through the cigarettes differed. The 70cc puff had almost twice the delivery of a normal puff. Samples of TPM were dissolved in DMSO (1 ml DMSO/10 mg TPM) for S/M testing.

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1. Kinser, R. Notebook No. 8137, pp. 17-20.

2. Morgan, R. Notebook No. 8102, pp. 77-80.

3. Lambert, E. Notebook No. 8050, p. 36.

4. Tafur, S. 6908 Monthly Progress Report. Monthly Progress Report 84-236; 1984 August 15.

5. Petri, D. Notebook No. 7701, pp. 153, 155, 162.

6. Levins, R. Notebook No. 8109, pp. 27-29.

7. Hellams, R. Notebook No. 7700, pp. 196-197.

8. Hellams, R. D. Six model cigarettes' impaction trap yield, filter efficiency data, and calculated contribution of varied blend components to the IT MS CSC delivery of a blended cigarette. Memo to Dr. E. B. Sanders; 1984 August 21.

S. Tafur

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CHARGE NUMBER: 8101

PROGRAM TITLE: Cigarette Testing Services

SECTION LEADER: Joyce F. Stargardt

PERIOD COVERED: August, 1984

## I. METHODOLOGY

### A. BORGWALDT CYLINDER VOLUME (H. Early)

Objective:

To computerize the data acquisition of the Borgwaldt CV analysis using an IBM-PC/XT.

Status:

A utility has been added to the cylinder volume data acquisition system to allow the manual entry of oven volatiles data. This utility also uses the oven volatiles data for correcting the cylinder volume data to a 12.5% moisture level.

A second utility was added to the system to produce data files containing the cylinder volume data in the format required by the Laboratory Automation System (LAS).

The LAS formatted files can then be transferred to the DEC 2060 at PM700 via a file transfer protocol called Kermit. The data can then be submitted to the database.

### B. RECONSTITUTED LEAF (RL) ATTRITION STUDY (N. Ryan)

Objective:

To determine the precision of microscopic blend separation in various sieve fractions.

Status:

Twelve samples were prepared by Mr. Bob Rogers. The samples consisted of known amounts of RCB and RL of various sieve fractions, combined in a typical Marlboro blend. The RL and RCB tobacco components were microscopically separated from these blends in order to determine the precision of this manual separation process. The RSD of these separations, at the 2-sigma level, was ±2% on the prepared samples.

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C. LABORATORY EFFICIENCY INVESTIGATIONS (F. Dawson)

Objective:

To increase the efficiency of ripping cigarettes and filters.

Status:

An electric ripper, purchased from S.O.D.I.M., has been put into use in the Physical Testing Lab. It provides longitudinal slitting of cigarettes, with or without filters attached, and slitting of filters alone. It gives clean cuts, is adjustable to different cutting depths, and can handle up to 100 rods/minute. A second ripper has been ordered for the Gas Chromatography Lab. (See August 14, 1984 memo to J. E. Wickham).

II. BRAND INTRODUCTION

A. LIGGETT GROUP

Generic Products Corporation is distributing Full Flavor Quality 85 and 100 cigarettes. This generic product is manufactured by the Liggett Group.

III. MEMOS AND REPORTS

1. C. F. Dawson to J. E. Wickham, "Automation of Tube Filling for the Nicotine and Water Laboratory," August 8, 1984.

2. C. F. Dawson to J. E. Wickham, "Cigarette and Filter Ripping," August 14, 1984.

3. N. R. Ryan to J. F. Stargardt, "Microscopic Blend Separation - RL Attrition Study," August 10, 1984.

4. G. V. Carter to J. F. Stargardt, "Conditioning for Cylinder Volume (CV) - Cigarettes vs. Loose Filler," August 6, 1984.

5. B. S. Mait to Distribution, "Interim C.I. Highlights Report," August 30, 1984.

6. C. F. Dawson to J. E. Wickham, "Coal Strength of Domestic Brands," August 21, 1984.

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Joyce F Stargardt

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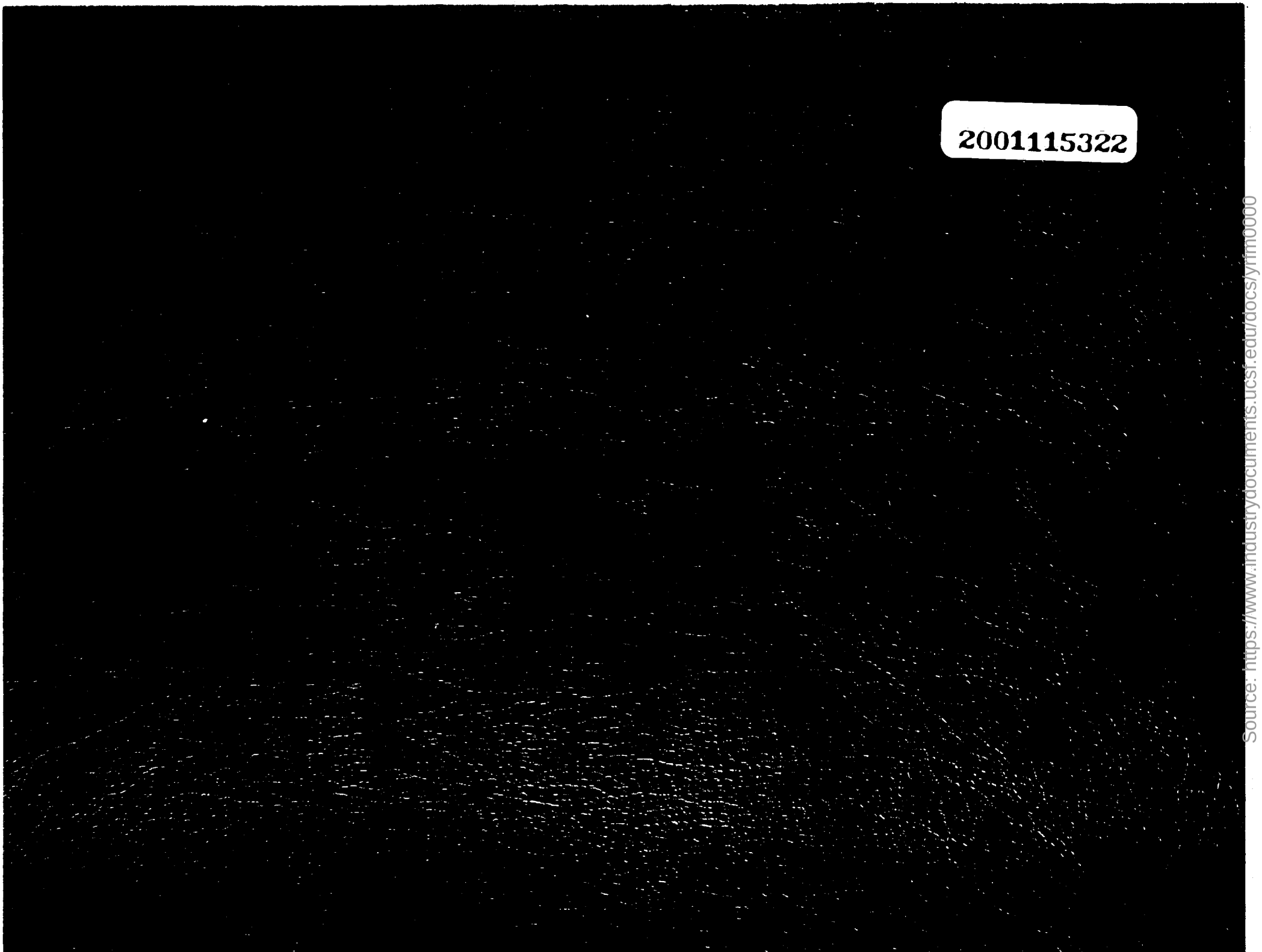

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